Effect of salt loving bacterial isolates on the enzymatic activity of the soil of Indian Sundarbans

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

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IN
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CERTIFICATE

This is to certify that the thesis entitled "**Effect of salt loving bacterial isolates on the enzymatic activities of the soil of Indian Sundarbans** " has been submitted by **Triparna Mitra** , a student of final year M.Sc. in Microbiology, 2023-2025 (Registration Number-230010219007), (Enrollment ID-2322211001021) of **Sister Nivedita University, Kolkata**. This project work is the outcome of the work carried out by her under the guidance of Prof. Pabitra Banik and Ms. Sreemoyee Mitra from 01-01-25 to 30-04-25 in the laboratory of the **Agricultural and Ecological Research Unit (AERU), Indian Statistical Institute (ISI), Kolkata, India**. We believe that the thesis fulfils all the necessary requirements for the award of Master of Science in Microbiology.

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TO WHOM IT MAY CONCERN

This is to certify that the project report titled “Effect of salt loving bacterial isolates on the Enzymatic activity of the soil of Indian Sundarbans", submitted by Ms. Triparna Mitra (Registration No. 230010219007, Enrolment No2322211001021) to the Department of Microbiology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a bona fide record of research conducted by her for the partial fulfillment of the requirements for the Master of Science degree.

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DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled – "Effect of salt loving bacterial isolates on the enzymatic activity of the soil of Indian Sundabrbans" in partial fulfilment of the requirements for the award of the degree of Masters of Science in Microbiology is an authentic record of my own work carried out during the period from January to April under the supervision of Prof. Pabitra Banik, Department of Agriculture and Ecological Research Unit, Indian Statistical Institute, I acknowledge that I have read and understood the UGC regulations, 2018 (Promotion of Academic integrity and Prevention of Plagiarism in Higher Educational Institutions).

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Abbreviation

Abbreviation	Full form
pH	Potential Of Hydrogen
EC	Electrical Conductivity
OC	Organic Carbon
CEL	Cellulase Activity
DHA	Dehydrogenase Activity
POX	Peroxidase Activity
DNS	3,5-dinitrosalicylic acid
TTC	2,3,5-triphenyltetrazolium chloride.

Abstract

The study concentrates on the enzymatic activity of the bacterial enumerated soil of the Indian Sundarban and also the organic carbon measurement. The soil samples were collected from the different location within Nimpith (Sundarban).

This research explored how different soil treatments could improve soil health in salty environments. Throughout the study, the focus was on several important soil properties. The pH values across all experimental groups remained within a moderately alkaline range (8.02–8.72) and showed minimal variation, indicating that the treatments did not significantly alter soil pH. Electrical conductivity was generally reduced in the treated groups compared to the control, reflecting a decrease in soil salinity. Which is essential for supporting healthy plant growth.

Several treatments led to a marked increase in soil organic carbon content, suggesting enhanced soil fertility and improved potential for sustainable crop production.

Phosphorus levels were consistently lower in treated soils, which may help mitigate the risk of nutrient runoff and environmental eutrophication.

Most treatments resulted in peroxidase and glucose enzyme activities that were comparable to or lower than the control, with some treatments demonstrating enhanced enzymatic activity related to cellulose degradation.

Overall, the study highlights that thoughtful soil management can help counteract the negative effects of salinity, leading to healthier soils and more sustainable farming in challenging conditions.

Improved soil conditions can support a wider variety of crops and beneficial soil organisms, fostering a more balanced and diverse ecosystem.

The promising results from certain treatments suggest that further research and application of salt-tolerant bacteria could help reclaim and improve saline soils in other regions, making previously unproductive land viable for agriculture.

Continued use of organic amendments and targeted microbial treatments could gradually restore degraded soils, making them more fertile and resilient to future environmental stresses.

With the threat of rising soil salinity due to climate change, these approaches offer practical solutions for sustaining food production. Future studies could focus on large-scale field trials, economic assessments, and developing easy-to-use products for farmers, ensuring these benefits reach more communities

Keywords:

Soil tolerant bacteria, Soil enzymatic activity, Indian Sundarbans, Salinity stress, Cellulase, Dehydrogenase, Peroxidase, Soil health, Soil pH, Electrical conductivity, Organic carbon, Microbial diversity, Soil physiochemical properties.

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Introduction

Soil is much more than just earth beneath our feet—it's a vibrant blend of minerals, moisture, air, and a whole community of living organisms, all working together to support life above ground. All these ingredients come together to create the foundation for healthy plant life and thriving ecosystems. It forms the uppermost layer of the earth, providing habitat and shelter for various vibrant organisms and supporting numerous ecological communities. The organic matter makes only 5%, filling voids, water and air takes up to a 50% and minerals taking the lead with 45%.

Soil is made through weathering and erosion, along with the decomposition of organic material due to climate, organisms and topography (Williams, Friedl 2023). It serves a critical role in water storage, agriculture, nutrient cycling and modifying the atmosphere.

The soil is home to a wide variety of tiny living organisms, including bacteria, fungi, archaea, and protozoa. All of these are known as microbes.
An astonishing amount of them, up to billions, are contained in just 1 gram of soil.

Microbes also help in soil fertility through recycling nutrients, decomposing residues and breaking down organic matter.

The rhizosphere or the placed around plant roots is vital because, it aids the plant in further growth, recovery and tolerance. Plants symbiotically team up with phytomicrobes, augmenting growth and tolerance against stress and infact disease.

Active microbes and healthy oil boost agricultural output by improving nutrient availability, making the soil more fertile and warding off pests and disease. Soil serves as much more than just a medium for plant roots; it is a vital resource that supplies plants with the nutrients and water they need to thrive. The structure of soil offers a stable foundation where plant roots can firmly anchor themselves and extend outward as they seek water and nutrients. This anchorage not only stabilizes the plant but also enables roots to absorb essential minerals and moisture, both of which are crucial for initiating and sustaining key metabolic functions.

These processes are fundamental to plant growth, energy production, and longterm survival.

In this way, soil acts as both a support system and a life-sustaining source, directly influencing the health and vitality of plants.

Soil gives nutrients, waters, and a stable place where roots can fixate and work optimally for development and sustenance. Fertile soil inhabited by microorganisms is extremely beneficial for agricultural production as it increases the nutrient supply and inhibits pathogens and pests. Microbial activities help to mitigate climate change through sequestering carbon in soil. (Front. Soil Sci., 11 April 2022 Sec. Soil Biogeochemistry & Nutrient Cycling)

Soil preserves temperature, controls flooding, and minimizes erosion which helps whole ecosystems. It also stores water, filters it, and purifies it as well controlling water movement to aquifers and rivers while reducing contamination.

The structure of soil affects the water retention and drainage to maintain the required moisture levels for plants while avoiding waterlogging. Organic matter protects soil from erosion, retains long-term productivity, and binds particles together. Moreover, soil contains carbon which assists in lowering the emission of greenhouse gases while preserving fertility. (MG kibblewhite, K Ritz, MJ Swift 2007)

Soil enzymes are natural substances that help accelerate the decomposition of organic materials in the soil, making essential nutrients available for plants. Soil enzymes are generated by a variety of sources, including microorganisms, the roots of plants, soil-dwelling animals, and the breakdown of organic material. Nutrient cycling and soil structure depend on each enzyme having a particular solvent. Soil enzyme activity improves nutrient cycling (Carbon, Nitrogen, Phosphorus) enhancing soil fertility and crop yield, thus, soil productivity (Li Wang, Chantal Hamel 2023).

Also, alleviate climate change and land management impacts on soil health, serve as proxies for microbial soil life and soil health, and strategy to manage ecosystem productivity by propelling enzyme activities related to the carbon and nitrogen cycles. Soil enzymes help break down organic materials, making nutrients available for plants and helping the soil store carbon. By carefully managing these enzymes, we can keep our soils healthy and support farming and environmental practices that are good for the planet.

Soil enzymes are the result of diverse biological and chemical factors that are responsible for their activity during soil nutrient cycling and soil wellbeing:

Microorganisms like bacteria, fungi, and actinomycetes are the primary producers of soil enzymes. Forthcoming microbial activity liberates such enzymes as phosphatases, protease, urease and cellulase which drive the nutrient cycling procsses (carbon, nitrogen, and phosphorus cycle).

Root Exudates: Roots Exudates enzymes into the rhizosphere and enable nutrients to enter the plant. For example, specific phosphatase enzymes help make phosphorus more available for plants, while proteases assist in the release of nitrogen.

Soil Fauna: The soil cartoon can also ilicite enzymes like cellulase and chitin through their alimentary processes. Enzymes produced during the decomposition of cadavers play an important role in breaking down organic matter in the presence of oxygen. These enzymes help speed up the process of converting complex materials into simpler substances, making nutrients more accessible for soil organisms.
Organic Residues: Primary and secondary plant decomposing along with mechanical corpse will liberate certain insoluble enzymes into the organic soil matrix. For example, plant sources are known to contain enzymes like glucosidases, while proteins are typically broken down by enzymes called proteases. (Jokūbas Daunoras, Ranata Gudiekaute 2024).

Stabilized Enzymes: Enzymes are often attached to the particles of clay, humus and humus in clay complex while retaining full activity post demise of the source organisms (Rev. Bras. Ciênc. Solo 45 2021).

2.**Review literature**

Before reviewing the research in literature, it will be necessary to delimit and synthesize the literature separately in terms of source and field. This has been google scholar based review implying the selected papers have been chosen widely and a broad selection of relevant academic work. Most of these have focused on Enzymatic Activity of Soil and Soil ecology, physiochemical properties, organic carbon of soil samples. Here are the summaries of different mechanism, implying in these researches.

No.	Year	Author	Title	Brief description	References
1	2021	Ghosh, P., & Mandal, S.	Phosphorus Dynamics in Indian Sundarbans Soils: Influence of Salinity and Microbial Activity	Investigated the fate of phosphorus in highly saline Sundarbans soils, analyzing how salinity impacts its availability and the role of microbial communities in phosphorus cycling	Ghosh & Mandal 2021
2	2015	Das, B., & Adhikari, S,	Soil Organic Carbon Stocks and Sequestration Potential in Degraded Mangrove Forests of Indian Sundarbans	Assessed the levels of organic carbon in different mangrove areas, discussing the impact of degradation on carbon dynamics and the role of soil biological processes.	Das & Adhikari 2015
3	2014	Manna, M., & Roy, S.	Dehydrogenase Activity as an Indicator of Microbial Activity in Salt-Affected Soils	A comprehensive review on the utility of dehydrogenase enzyme activity as a robust indicator of overall microbial metabolic status in soils subjected to salt stress	Manna & Roy 2014
4	2022	Xiaoyan Wang, Qiang Chen, et al.	Composition and Diversity of Soil Microbial Community Associated with Different Land Use Types	The authors examine how environmental factors and land use influence soil microbial diversity, using high-throughput sequencing and statistical analyses to relate microbial changes to soil properties	Wang X, Chen Q, et al. (2022).
5	2006	Datta, D., & Datta, S.	Soil Enzyme Activities in Relation to Soil Physicochemical Properties in the Sundarbans Biosphere Reserve	Investigated the relationship between various soil enzyme activities (including dehydrogenase) and key physicochemical properties like pH and EC in Sundarbans soils.	Datta & Datta (2006)
6	2023	Zhenzhen Wang, Xiaoyan Zhang, Yanan Li, Xuefeng Wang, Zhenhua Yu	Soil enzyme activities and their relationship with soil organic matter in saline soils	Found strong positive correlations between organic carbon and activities of peroxidase and dehydrogenase in saline soils.	Wang et al. (2023)

7	2021	Jupinder Kaur and Gurpreet Kaur	Dehydrogenase Activity as a Biological Indicator of Soil Health	Reviews dehydrogenase as a sensitive indicator of soil health, linking its activity to microbial population and ecosystem changes.	Kaur et al. (2021)
8	2023	Singh, Anil Kumar; Kumar, Ravindra; Kumar, Sunil; Kumar, Amit; Kumar, Sushil; Kumari, Poonam.	Quantification of soil dehydrogenase enzyme activities in long-term fertilizer and manure experiments	Examines the impact of long-term fertilizer and manure use on dehydrogenase activity in rice and wheat soils, showing higher activity with balanced fertilization and organic amendments.	Singh et al. (2023)
9	2014	Wolińska, Agnieszka; Stępniewski, Janusz; Szymańska, Marek. 2014	Soil microbial communities and dehydrogenase activity depending on soil properties	Discusses dehydrogenase as a sensitive bioindicator of soil fertility, affected by soil properties and management.	Wolińska et al. (2014)
10	2010	Kaczyńska, Grażyna; Boros-Lajszner, Anna; Wyszkowska, Jolanta.	Dehydrogenase activity of forest soils depends on the assay used	Compares methods for measuring dehydrogenase activity in forest soils and correlates results with soil properties.	Kaczyńska et al. (2010)
11	2010	Sahu, R.K. & Singh, M.	Dehydrogenase Activity in Natural and Mine Soil	Investigates dehydrogenase activity as an indicator of nutrient cycling and microbial activity in natural vs. mine-affected soils.	Sahu & Singh (2010)
12	1998	Ross, D.J.	Soil oxygen status and dehydrogenase activity	Explores the essential role of dehydrogenases in soil organic matter oxidation, affected by soil oxygen status.	Ross (1998)
13	2018	Sunil Kumar, Ajay Kumar, Ravindra Singh, Sangeeta Sharma, and Rajesh Kumar	Soil dehydrogenase activity as influenced by land use and management	Reviews the effects of land use changes and agricultural practices on dehydrogenase activity and soil microbial health.	Kumar et al. (2018)
14	2013	Jyoti Singh, Nidhi Batra, Ramesh Kumar Sobti	Optimization of Cellulase Production from Bacteria Isolated from Soil	Investigates optimal conditions for bacterial cellulase production from soil isolates, emphasizing industrial applications and microbial diversity.	Singh et al. (2013)
15	1994	Karl Alef, Paolo Nannipieri	Cellulase activity of soils	Describes precise methods for cellulase assay in soils, examining environmental factors influencing enzyme activity.	Alef & Nannipieri (1994)

16	2017	Li Zhang, Hongyu Chen, Xiaoyan Liu	Cellulase activity and microbial community in soils with different organic amendments	Examines the effect of various organic amendments on soil cellulase activity and associated microbial community changes.	Zhang et al. (2017)
17	1971	R.G. Burns	A rapid method for measuring cellulase activity in soils	Introduces a laboratory method for quickly measuring cellulase activity in soils, facilitating large-scale assessments of soil enzyme function.	Burns (1971)
18	1994	Environmental Protection Agency (Corvallis, OR)	Interferences, limitations and an improvement in the extraction and assessment of cellulase activity in soil	Describes a practical modification of the DNS reducing-sugar method for more reliable cellulase activity measurement in diverse soils, improving reproducibility and ecological relevance.	EPA (1994)
19	2024	Andreea Buda, Andrei Mihai Mihăilescu, et al	Role of Soil Microbiota Enzymes in Soil Health and Activity Changes: A Review	Reviews the main groups of microbial enzymes in soil (including cellulase, peroxidase, dehydrogenase), their ecological roles in carbon cycling, and how environmental and management factors affect their activity.	Buda et al. (2024)
20	2023	Science Direct Topics (Editorial)	Soil Enzymes – an overview	Overview of major soil enzymes (cellulase, dehydrogenase, peroxidase, etc.), their microbial origins, and their roles in nutrient cycling and soil health	Science Direct Topics (2023)
21	2021	Roseli Aparecida de Souza, Lucimara Aparecida dos Santos, et al	Contribution of enzymes to soil quality and the evolution of research in Brazil	Review highlighting the role of soil enzymes (including cellulase, peroxidase, dehydrogenase) in soil quality, nutrient cycling, and agricultural sustainability, with a focus on Brazilian research and advances	Souza et al. (2021)
22	2023	J. Zhang, Y. Liu, et al.	Soil enzyme activities, soil physical properties, photosynthetic characteristics, and crop yield	Examines the relationship between soil enzyme activities, microbial biomass, and soil organic carbon, finding consistent trends and highlighting the importance of enzyme activity for soil fertility and crop productivity.	Zhang et al. (2023)

23	2023	Y. Chen, J. Zhang, et al.	Using enzyme activities as an indicator of soil fertility in grassland	Reviews the use of soil enzyme activities as indicators of soil fertility, discussing their relationship with microbial biomass, soil properties, and management practices in grassland ecosystems.	Chen et al. (2023)
24	2021	Monika Krzyżak, Magdalena Gondek, et al.	Enzymatic Activity and Physicochemical Properties of Soil Profiles of Different Land Use	Examines β-glucosidase, dehydrogenase, and other enzyme activities along soil profiles, relating them to C, N, and P cycles and land use.	Krzyżak et al. (2021)
25	2020	Zhiwei Wang, Xuefeng Li, et al.	Soil enzyme activities and their relationship with soil properties in a degraded grassland	Studies the relationship between soil enzyme activities and soil properties in degraded grassland, highlighting the role of enzymes in ecosystem restoration.	Wang et al. (2020)
26	2018	R.G. Burns, J.L. DeForest, J. Marxsen, et al.	Soil enzymes in a changing environment: Current knowledge and future directions	Comprehensive review of soil enzyme research, including cellulase, peroxidase, and dehydrogenase, and their responses to environmental change and land management.	Burns et al. (2018)
27	2013	R.G. Burns, J.L. DeForest, et al.	Measuring phenol oxidase and peroxidase activities with pyrogallol as substrate in soil	Describes a standardized method for measuring peroxidase activity in soil using pyrogallol as a substrate, highlighting the enzyme's role in mediating biogeochemical processes, including carbon and nitrogen cycling.	Burns et al. (2013)
28	2024	Y. Chen, J. Zhang, et al.	Evaluating the Enzyme Activities and Soil Physicochemical Properties in Halophyte and Saline Soils	Assesses peroxidase activity among other enzymes in halophyte and saline-sodic soils, linking enzyme activity to organic carbon content and soil salinity, and highlighting the role of peroxidase in saline soil health.	Chen et al. (2024)
29	2009	J. L. DeForest	Phenol oxidase, peroxidase and organic matter dynamics of soil	Demonstrated that peroxidase activity generally increases with soil pH across ecosystems, influencing organic matter decomposition and carbon cycling.	DeForest (2009)

30	1996	Gianfreda, L., Bollag, J.M.	Influence of Natural and Anthropogenic Factors on Enzyme Activity in Soil	Discusses how environmental factors affect soil peroxidase activity and assay reliability.	Gianfreda, L., & Bollag, J.M. (1996).
31	2013	Wang, Q., Wang, S.	Soil Enzyme Activities and Their Relationship with Soil Organic Matter	Explores relationships between peroxidase activity (guaiacol method) and soil organic content.	Wang, Q., & Wang, S. (2013).
32	1994	Dick, R.P.	Soil enzyme activities: Indicators of soil quality	Discusses dehydrogenase activity as a reliable indicator of soil microbial function and health.	Dick, R.P. (1994).

3. Material And Methods

3.1 Study Area and Procedure for applying bacterial Solution to soil samples:

Materials Required

- Pots (sterile)

- Soil samples from Nimpith

- Bacterial strains

- Greenhouse chamber

- Water (distilled)

- Grinder (for soil grinding)

- Incubator (set at 37°C)

Experimental Procedure Soil

Preparation:

Soil samples were first collected from Nimpith, a small town positioned in the delta region of the Sundarbans, which lies within the extensive Ganges Delta. The soils in this area are generally a mix of clay, sand, and silt, and are regularly influenced by tidal flooding and varying levels of salinity due to their proximity to both river and sea. With its low elevation and closeness to the mangrove ecosystem, Nimpith serves as an ideal location for examining the unique soil properties that define the Sundarbans delta.

Figure 1: Map of Sundarban

Figure 2: Delta region of Sundarban

The soil samples were gently sifted to eliminate any stones and unwanted debris. Afterward, the soil was mixed thoroughly to achieve a uniform consistency throughout. Each experimental pot was filled with an equal amount of soil, specifically 200 grams per pot, to maintain consistency across all treatments.

Preparation of Bacterial Solution:

Bacterial strains were cultured in a suitable broadcast medium until they reached a cell density exceeding 10^8 CFU/mL.

Once the desired concentration was achieved, the bacterial cultures were harvested and suspended in sterile water or buffer to prepare the bacterial suspension.

A set amount of the bacterial solution (50 mL) was poured into each pot filled with soil. For the control group, instead of the bacterial solution, the same amount of sterile water-without any bacteria-was added to the pots. This helped ensure that any differences observed were due to the bacteria, not just the addition of liquid. The bacterial suspension was gently mixed into the soil to ensure even distribution, taking care not to disturb the soil structure excessively.

Incubation:

All pots were placed in a controlled environment, either a greenhouse or a growth chamber, and maintained at a constant temperature of 37°C for a period of 90 days. Soil moisture was kept at field capacity by regular watering, with moisture levels checked every 2–3 days. Care was taken to prevent water from accumulating around the roots, unless anaerobic conditions were specifically required for a particular treatment.

Throughout the incubation period, the pots were regularly inspected for signs of fungal growth, pest infestations, or any other abnormal features. Detailed logs were maintained, recording temperature, humidity, and other relevant environmental parameters. Where appropriate, samples were periodically collected at 30- or 60-day intervals to monitor changes in soil bacterial populations or other soil properties

Collection and Processing of Soil Samples after Incubation:

After 90 days, the soil from each pot was taken out and left to dry in the air overnight. Once the soil had dried, it was kept at room temperature until it stopped losing weight, making sure all the moisture was gone. Once fully dried, the soil was ground into a fine powder using a grinder and passed through a 2 mm sieve. The processed samples were subsequently stored in sealed containers

Figure 3: Soil Samples After Treatment

3.2 Measurement of Soil PH And Electrical Conductivity:

Soil pH showed whether the soil was more on the acidic or alkaline side, based on how many hydrogen ions were present in the soil's water. This was an important factor because it affected how easily plants could take up the nutrients they needed to grow well.

To find out the soil's pH, samples were mixed with water, and the amount of hydrogen ions in the mixture was measured. Knowing the pH helped to understand how well the soil could support different kinds of plants.

Sample Preparation

A 10-gram portion of air-dried, sieved soil was weighed and transferred into a clean 50 mL beaker. Subsequently, 25 mL of distilled water was added to the soil, maintaining a consistent ratio for all samples.

Mixing and Equilibration

The mixture was stirred to ensure even dispersion of soil particles. The mixture was gently stirred at regular intervals for a total of 30 minutes. After mixing, the beaker was left undisturbed for one hour to allow the soil particles to settle and the solution to reach equilibrium.

Calibration of pH Meter

Before taking any measurements, the pH meter was standardized with buffer solutions having pH values of 4.0, 7.0, and 9.2. The electrode was rinsed with distilled water between each calibration and sample measurement to avoid contamination.

pH Measurement

Once the suspension had settled, the pH electrode was carefully inserted into the supernatant above the settled soil. The pH reading was allowed to stabilize before being recorded for each sample.

Electrode Maintenance

After each measurement, the electrode was thoroughly rinsed with distilled water to maintain accuracy for subsequent samples.

Electro conductivity Test:

Principle

Electrical conductivity (EC) indicates how well a soil solution carries electricity, revealing the amount of dissolved salts affecting its salinity

Reagents and Calibration

For calibration purposes, a 0.01M potassium chloride (KCl) solution was employed as the standard reference. This solution typically exhibited an electrical conductivity value in the range of 1.412 to 1.413 dS/m at a temperature of 25°C. To prepare the 0.01M KCl standard, 0.746 grams of potassium chloride were accurately weighed and dissolved in one litre of distilled water. Prior to each series of measurements, the conductivity meter was calibrated using this standard solution to ensure precise and reliable results.

Procedure

A 10-gram sample of air-dried soil was accurately weighed and placed into a clean beaker.

To this, 50 milliliters of distilled or deionized water were added, maintaining a soil-to-water ratio of 1:5.

The mixture was then shaken well for about 30 minutes to make sure all the soluble salts in the soil dissolved completely.

The suspension was allowed to settle undisturbed for 4 hrs to obtain a clear soil extract for analysis.

Prior to measurement, the electrical conductivity (EC) meter was calibrated with the 0.01M KCl standard solution at 25°C to ensure accuracy.

The electrode was thoroughly rinsed with distilled water before being carefully immersed in the clear soil extract, taking care to avoid disturbing any settled sediment.

Once the reading stabilized, the EC value was recorded, with adjustments made for temperature to standardize results at 25°C if required. The final electrical conductivity values were reported in deciSiemens per meter (ds/m), which is equivalent to miliSiemens per centimeter (mS/cm).

3.3 Determination of Organic carbon in soil

Principle

The Walkley-Black method, one of the most used today, measures organic carbon in soils based on its virtual mineralization through wet chemical oxidation, which involves potassium dichromate (K2Cr2O7) in concentrated sulfuric acid (H2SO4). The acid facilitates heat and sulfonates the organic framework which is oxidized into carbon dioxide (CO2) gas. During this reaction, dichromate ions ($Cr_2O_7^{2-}$) interact with the carbon produced, which must be preserved.

This undissolved governorate is assesses through back titration with Ferrous Ammonium salt, using diphenylamine to mark the end of the reaction. While the rest is reached by calculation, tantamount remains Cr ammonium diphosphate which permits computation of the carbon content within the soil particle samples. Because the method typically oxidizes about 75-90% organic argent, a correction factor (referred to as 1.3) can be used for total organic carbon estimates is attained – which depend on opened samples is nearly accurate.

Oxidation:

$$2K2Cr2O7 + 8H2SO4 + 3C \rightarrow 2K2SO4 + 2Cr2(SO4)3 + 8H2O + 3CO2$$

Titration reactions:

$$2FeSO4\ (NH4)2SO4.\ 6H2O + H2SO4 \rightarrow (NH4)2SO4 + Fe\ (SO4)3 + 3H2O$$

Materials-

Burrete, conical flask (500 mL), 10mL pipette, measuring cylinder, Beaker, volumetric flask.

Reagents:

1. 1 N potassium dichromate (K2Cr2O7): Dissolved 49.5 g in 500ml distilled water in volumetric flask.

2. 0.5 N Ferrous Ammonium Sulphate (FAS): Dissolved 98g FAS in 500 ml distilled water, add 10mL of conc.H2SO4

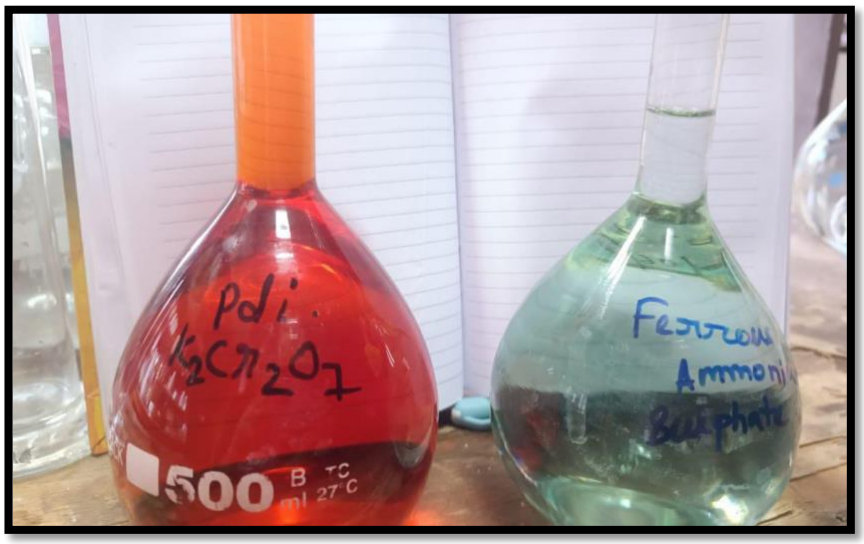


Figure 4: Potassium dichromate solution and Ferrous Ammonium Sulphate solution

To prepare the diphenylamine indicator, A total of 0.5 grams of diphenylamine was dissolved in 100 millilitres of concentrated sulfuric acid. The solution was gently stirred until all of the diphenylamine had fully dissolved.

Concentrated H2SO4

Orthophosphoric Acid

Procedure:

Approximately 1 g of air-dried, sieved soil from the each samples were weighed into 500 mL conical flasks using. Then 10 mL of 1 N potassium dichromate solution was gently added to the flasks and the flasks were swirled to mix the contents. Immediately afterward, 20 mL of concentrated sulfuric acid was carefully introduced in a slow stream in to the flasks, and the mixture was swirled to ensure thorough mixing. The flasks were subsequently left undisturbed at room temperature for 30 minutes to ensure complete digestion of the mixture.

Once the digestion process was complete, 4 mL of phosphoric acid was carefully added to each flask. The contents were then diluted with approximately 200 mL of distilled water to cool and further dilute the solution. Next, three to four drops of diphenylamine indicator were carefully introduced into the solution, allowing the color change to be observed.

The mixtures were then titrated with 0.5 N ferrous ammonium sulfate (FAS) solution, added slowly until the blue color transitioned to green, indicating the endpoint.

A blank titration was also performed using the same procedure but without the soil sample, to ensure accuracy and allow for correction of reagent volumes.

Volume of FAS used for the sample titration and compared it to the blank titration (without soil). Find out how much organic carbon is present by using this formula: %organic carbon= 10× (Vblank –Vsample) /blank × 0 .003 ×100 /weight of soil (1g)

Vblank= volume of FAS for blank (mL)

Vsample=volume of FAS for sample (mL)Each millilitre of 1N $K_2Cr_2O_7$ corresponds to 0.003 grams of carbon that has been oxidized.

The Walkley-Black method was used to determine soil organic carbon by oxidizing the organic carbon present in the soil with chromic acid, which was produced from the reaction between potassium dichromate and sulfuric acid. After the oxidation reaction, the remaining unreacted dichromate was measured by back-titration with ferrous ammonium sulfate, using diphenylamine as an indicator to identify the endpoint. This method enabled the measurement of the amount of oxidizable organic carbon present in the soil samples.

Figure 5: Soil organic carbon samples determination by The Walkley-Black method

3.4 Estimation of Available Phosphorus:

To measure available phosphorus in neutral to alkaline soils, the Olsen method is widely used. This approach starts by shaking a soil sample with a sodium bicarbonate solution (0.5 M, pH 8.5), which helps release phosphorus that plants can use by breaking its bonds with soil particles. After extraction, the filtered solution is treated with ammonium molybdate in an acidic medium, causing a chemical reaction that forms a blue-coloured compound. Adding stannous chloride further intensifies this blue color, making it easier to measure the phosphorus content with a spectrophotometer. The richer and deeper the blue color that appears in the solution, the more phosphorus is available in the soil sample. This color change makes it easy to estimate the phosphorus content, as a stronger blue indicates a higher amount of phosphorus that plants can use.

Materials Used

Soil samples
Sodium bicarbonate ($NaHCO_3$), analytical grade
Ammonium molybdate reagent
Stannous chloride ($SnCl_2$) solution
Deionized or distilled water
1 M sodium hydroxide (NaOH) for pH adjustment
Whatman No. 42 filter paper or equivalent
125 mL wide-mouth shaking bottles
Volumetric flasks (25 mL)
Shaker
Spectrophotometer

Preparation of Reagents

Sodium Bicarbonate Extractant (0.5 M, pH 8.5):

84.01 g of $NaHCO_3$ was dissolved in approximately 1.5 L of deionized water in a 2 L volumetric flask. The pH was adjusted to 8.5 using 5 M NaOH while stirring. The solution was then diluted to exactly 2 L with deionized water and mixed thoroughly.

Ammonium Molybdate Reagent:

15 g of ammonium molybdate was dissolved in 400 mL of distilled water. Then, 350 mL of concentrated sulfuric acid was added slowly with constant stirring. The mixture was diluted to 1 L with distilled water and stored in a plastic bottle.

Stannous Chloride Solution:

2 g of stannous chloride dihydrate was dissolved in 100 mL of a 1:1 mixture of concentrated hydrochloric acid and distilled water. This solution was prepared fresh daily to ensure stability.

Extraction Procedure

1. For each soil sample, 5 g of air-dried, sieved soil was weighed into a 125 mL wide-mouth shaking bottle.
2. 100 mL of the 0.5 M sodium bicarbonate extractant was dispensed into each bottle.
3. The bottles were sealed and shaken mechanically for 30 minutes at 180–200 oscillations per minute.
4. The suspension was filtered.

Colour Development and measurement:

1. A 5 mL aliquot of the filtrate was pipetted into a 25 mL volumetric flask.
2. First, 10 mL of ammonium molybdate reagent was introduced to the mixture, and then 1 mL of freshly prepared stannous chloride solution was added right after.
3. The mixture was topped up to 25 mL with distilled water, then left to sit for 10 minutes to allow the color to develop.
4. The intensity of the blue color formed was measured using a spectrophotometer at 880 nm. The phosphorus concentration was determined by comparing the absorbance with that of standard solutions treated similarly

Figure 6: Available Phosphorus Extraction from the soil samples

Preparation of Stock Solution

A primary phosphorus stock solution was prepared by dissolving 0.2195 g of potassium dihydrogen phosphate (KH_2PO_4, dried at 105°C) in distilled water and diluting to 1 litre. This solution contained 50 mg per Litre (50 ppm P). To prepare a secondary stock solution with a concentration of 100 ppm, I took 2 milliliters of the primary stock solution and carefully diluted it to a final volume of 1 litre using distilled water. This process ensured that the secondary solution reached the desired concentration.

Preparation of Working Standards by Serial Dilution

A series of working standard solutions with concentrations of 1, 5, 10, 20, 40, 60, 80, and 100 ppm were prepared from the 100 ppm secondary stock solution using serial dilution.

Each standard was prepared in a clean 10 mL volumetric flask. The required volume of the 100 ppm stock solution was pipetted into the flask, and distilled water was added to bring the total volume to 10 mL. Each solution was mixed properly.

Final concentration (ppm)	Volume of 100 ppm stock. (mL)	Final volume (ml)	Volume of distilled Water (ml)
100	10.0	10	0
80	8.0	10	2
60	6.0	10	4
40	4.0	10	6
20	2.0	10	8
10	1.0	10	9
5	0.5	10	9.5
1	0.1	10	9.9

Each standard was prepared in a clean 10 mL volumetric flask. The required volume of the 100 ppm stock solution was pipetted into the flask, and distilled water was added to bring the total volume to 10 mL.

Color Development and Measurement

In a 25 mL volumetric flask 5 ml of each standard solution was transferred. To this, 10 mL of ammonium molybdate reagent and 1 mL of freshly prepared stannous chloride solution were added.

The solution was then carefully topped up to the final volume with distilled water and mixed well to ensure uniformity. After mixing, the samples were left undisturbed for 10 minutes, allowing the blue color to fully develop before any measurements were taken.

The OD of the solution was measured at 880 nm wavelength by a spectrophotometer.

Assay of Soil Enzymatic Activity:

- Cellulase
- Dehydrogenase
- Peroxidase

Soil Enzyme Activity as an Indicator of Soil Health and Fertility

Soil enzyme activity is fundamental to the decomposition of organic matter and the cycling of nutrients, processes that are integral to maintaining soil quality and fertility. These enzymes, which originate primarily from soil microorganisms, plant roots, and decomposing organic residues, catalyze the breakdown of complex organic substrates into simpler compounds. This transformation not only facilitates the release of essential nutrients for plant uptake but also drives the biogeochemical cycling of key elements such as carbon, nitrogen, and phosphorus.

One of the most remarkable things about soil enzymes is how quickly they react when something in their environment changes-whether it's a shift in farming practices or fluctuations in weather conditions.

As a result, the measurement of soil enzyme activities has emerged as a sensitive and early indicator of shifts in soil health and fertility, often preceding detectable changes in other soil properties.

Among the diverse array of soil enzymes, several play particularly significant roles:

Cellulase is responsible for the hydrolysis of cellulose, a major structural component of plant cell walls. Through the breakdown of cellulose into simpler sugars, cellulase contributes to the soil carbon cycle and accelerates the decomposition of plant residues.

Dehydrogenase serves as a marker of overall microbial oxidative activity, as it is involved in the respiratory processes of soil microorganisms. Dehydrogenase activity takes place only inside living microbial cells, so it serves as a clear sign of the presence of active, living microbes in the soil.

Peroxidase helps break down tough organic substances like lignin and also plays a key role in detoxifying harmful compounds in the environment. Its activity supports the transformation of soil organic matter into forms that are more accessible to plants and microorganisms.

Soil enzymes often get a helping hand from their surroundings-they can attach themselves to bits of organic matter or clay in the soil. This connection not only shields them from breaking down too quickly but also helps control how active they are and how long they stick around. As a result, these enzymes can keep working steadily, playing their part in keeping the soil healthy and fertile.

Looking at how active these enzymes are gives us a clear window into the soil's overall biological health. Since they're so involved in breaking down organic material and recycling nutrients, tracking their activity helps us understand how well the soil is functioning and whether it's in good shape for supporting plant life.

The activity and persistence of these enzymes in soil are influenced by a variety of factors, including their origin, soil type, and interactions with the soil matrix. By serving as early indicators of changes in soil quality, these enzymes offer critical insights for sustainable soil management and the maintenance of soil fertility.

Cellulase Enzyme Activity

Principle:

Cellulase enzymes are responsible for degrading cellulose, which is a major structural component of plant cell walls composed of long chains of glucose molecules. In order to evaluate cellulase activity in soil, a commonly used method involves incubating soil samples with carboxymethyl cellulose (CMC) as a substrate. The breakdown of CMC by soil enzymes releases reducing sugars, which can be quantified using the dinitrosalicylic acid (DNS) assay. The intensity of the color formed during this reaction, measured at 540 nm with a spectrophotometer, serves as an indicator of the enzymatic activity present in the soil.

Materials Needed:

Soil sample (2.5 g)

Citric acid anhydrous and tri-sodium citrate dihydrate (for buffer)

Carboxymethyl cellulose (CMC)

DNSA (3,5-dinitrosalicylic acid)

Sodium hydroxide (NaOH)

Sodium potassium tartrate

Distilled water

Equipment:

Various types of glassware were used throughout the procedure, including test tubes, conical flasks, volumetric flasks, and centrifuge tubes.

Shaker, centrifuge, and incubator maintained at 37°C

Pipettes, hot water bath or oven

Spectrophotometer set to 540 nm

Preparing Buffers and Reagents:

Citrate Buffer:

To prepare the citrate buffer at pH 5, 1.754 grams of citric acid and 4.667 grams of tri-sodium citrate were thoroughly dissolved in distilled water. The mixture was then topped up with more liquid until it reached a total volume of 500 mL.

CMC Solution (1%, 150 mL):

1% CMC solution was made by dissolving 1.5 g of CMC in 149.5 mL of pre-warmed citrate buffer.

DNS Reagent (250 mL):

For the DNS reagent, 2.5 g of DNSA was dissolved in 60 mL of hot water (maintained at 60– 70°C). Separately, 2 M NaOH was prepared in 40 mL of water and added to the DNSA solution. Sodium potassium tartrate was dissolved in 150 mL of hot water and then combined with the previous mixture, making up a total volume of 250 mL.

Procedure:

Incubation:

Each test began by weighing 2.5 g of soil into a test tube. 5 milliliters of citrate buffer and an equal volume of the CMC solution were carefully added to each soil sample, ensuring thorough mixing to promote even interaction between the components. The contents were thoroughly mixed and incubated at 37°C for 30 minutes to allow enzymatic hydrolysis of CMC.

Termination of the Reaction:

Following incubation, the tubes were centrifuged to separate soil particles from the reaction mixture. To stop the enzymatic reaction, 3 mL of DNS reagent was added to each tube.

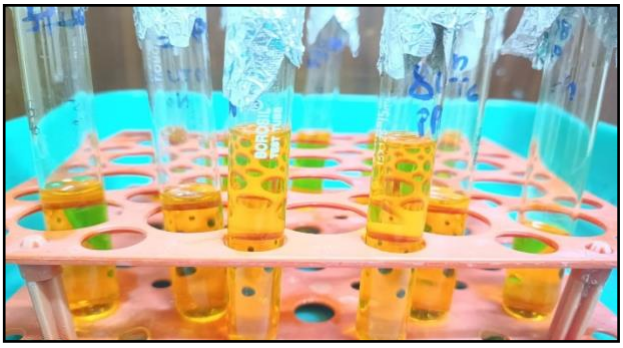


Figure 6: Termination of the Reaction using DNS reagent

Color Development:

The reaction mixtures were then transferred to a hot water bath set between 90 and 100°C and heated for 15 minutes, allowing the color to develop fully. After heating, the tubes were allowed to cool to room temperature.

Figure 7: Color development after heating the reaction mixture

Measurement:

The absorbance of each sample was measured at 540 nm using a spectrophotometer. The intensity of the color produced was directly proportional to the amount of reducing sugars released, and thus reflected the cellulase activity present in the soil samples.

Glucose Standard Curve Preparation and Calculation of Released Reducing Sugar

Glucose Stock Solution (1000 ppm or 1 mg/mL):

A quantity of 100 mg of anhydrous glucose powder was accurately weighed and transferred into a 100 mL volumetric flask. Distilled water was gradually added to the flask, and the glucose was dissolved completely by gentle swirling. Once the glucose had fully dissolved, the volume was made up to the 100 mL mark with additional distilled water, resulting in a stock solution with a concentration of 1000 ppm (1 mg/mL).

Preparation of Glucose Standards (200, 400, 600, 800 ppm)

Working standards of glucose at concentrations of 200, 400, 600, and 800 ppm were prepared from the stock solution using the dilution formula:

$$C1 \times V1 = C2 \times V2$$

V1= stock solution volume, V2 = final volume of working standard (usually 10 mL).

Preparation of the Glucose Standard

The calculated volumes of the stock solution were pipetted into separate 10 mL volumetric flasks, and each flask was then filled to the mark with distilled water to achieve the desired concentrations.

Glucose Standard Assay Using the DNS Method

For the assay, 1 mL of each glucose standard was transferred into individually labeled test tubes .3 milliliters of DNS reagent were added to each test tube, and the mixtures were stirred well to ensure proper blending. The tubes were then transferred to a boiling water bath, where they were heated for 15 minutes to allow the color to develop. Once the incubation period was complete, the tubes were taken out of the water bath and left to cool down to room temperature. The absorbance of each sample was measured at 540 nm using a spectrophotometer, with a reagent blank (containing all reagents except glucose) serving as the reference.

Points should be:

Peroxidase Enzyme Activity:

Principle:

The peroxidase enzyme uses hydrogen peroxide to oxidize guaiacol, resulting in a brown product. By monitoring the increase in absorbance at 465–470 nm, we can determine how active the peroxidase is in the sample-the faster the absorbance rises, the higher the enzyme activity.

Simplified Reaction:

Guaiacol + Hydrogen Peroxide → (Peroxidase) → Tetraguaiacol (brown) + Water

Materials

Soil sample (1gram per tube)

Acetate buffer (2.5ml per tube)

Guiacol solution (2ml per tube)

Hydrogen peroxide solution (1ml per tube)

Distilled Water

Equipment

Test tubes,

Conical and volumetric flasks,

Centrifuge tubes

Pipettes

Shaker

Incubator

Spectrophotometer (436nm)

Buffer and Reagent preparation:

Acetate Buffer preparation (pH ~5)

Sodium acetate (0.246 g) and acetic acid (0.180 g) were dissolved in distilled water and the volume was adjusted to 60 ml to obtain a 50 mM acetate buffer.

Preparation of 50 mM Guaiacol Solution

0.376 g of 99% guaiacol was dissolved in distilled water and the volume was made up to 60 ml to prepare a 50 mM solution.

Preparation of Hydrogen Peroxide Solution

0.41 ml of 30% H_2O_2 was diluted with distilled water to a final volume of 50 ml (49.59 ml water) to obtain the working solution.

Procedure:

0.3 g of soil was weighed for each assay tube.

2.5 ml of the buffer solution was added into each assay tube

2 ml of the guaiacol solution was introduced into each assay tube.

1ml of the diluted hydrogen peroxide solution was carefully added to each tube.

The tubes were shaken thoroughly and incubated at room temperature (approximately 30°C) for 45 minutes to allow the reaction to proceed.

Measurement

After the incubation, the absorbance was checked at 436 nm with a spectrophotometer.

A tube without soil was used as a blank to set the baseline for the instrument.

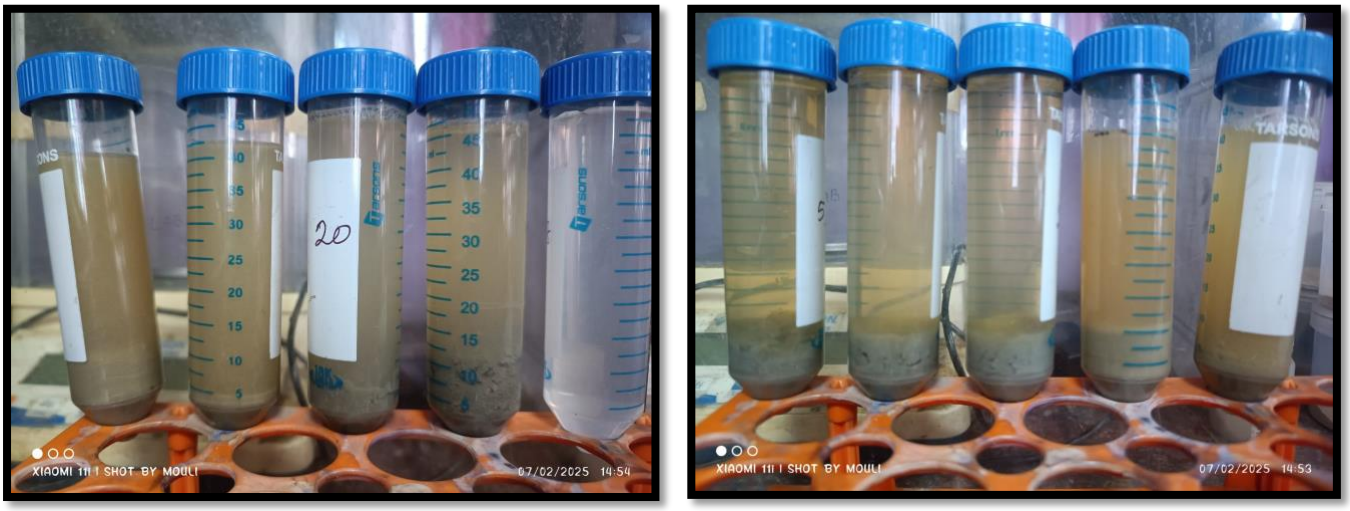


Figure 8: OD measurement of the soil samples after Incubation

Methodology for Measuring Soil Dehydrogenase Enzyme Activity:

Principle:

Dehydrogenase enzyme activity in soil was based on the ability of soil microorganisms to catalyze the oxidation of organic substrates by transferring hydrogen atoms to artificial electron acceptors, such as triphenyltetrazolium chloride (TTC). During this process, TTC was reduced to triphenyl formazan (TPF), a red-coloured compound. The amount of TPF formed was directly proportional to the dehydrogenase activity present in the soil, providing an indicator of overall microbial oxidative activity and soil biological health.

Buffer and TTC preparation

Phosphate Buffer preparation:

2.021 g of sodium phosphate dibasic dehydrate and 0.339 g of sodium phosphate monobasic monohydrate were weighed and dissolved in approximately 80 mL of distilled water. After pH adjustment, the solution was brought up to a final volume of 100 mL with distilled water and mixed thoroughly.

3% TTC solution preparation:

3 g of TTC powder were dissolved in 100 mL of distilled water. The solution was mixed until the TTC had completely dissolved.

Sample Preparation:

1 gram of soil was weighed and transferred into each test tube.

Calcium carbonate (0.2 g) was added to each tube.

Buffer Addition and Mixing

To each tube, 2.5 ml of phosphate buffer was added.

The mixture was thoroughly mixed to ensure even distribution of the reagents.

Substrate Addition

1 millilitre of 3% TTC (triphenyltetrazolium chloride) solution was added to each tube.

The contents were mixed again to ensure proper reaction.

Incubation

The tubes were incubated for 24 hours to allow the enzymatic reaction to proceed.

After incubation, 10 ml of methanol was added to each tube to stop the reaction and extract the coloured product.

The tubes were mixed thoroughly.

Centrifugation

The mixtures were spun in a centrifuge at 3000 rpm so that the soil particles would settle at the bottom, making it easy to collect the liquid on top.

Measurement

The optical density (OD) of the clear supernatant was measured at 485 nm using a spectrophotometer to determine dehydrogenase activity.

Figure 9: Collected supernatant after centrifugation to measure the Dehydrogenase activity

Preparation of the Dehydrogenase Standard Curve:

To construct the standard curve for dehydrogenase activity, a stock solution of formazan was first prepared. After carefully weighing out 0.1 grams of formazan, it was completely dissolved in methanol. The solution was then transferred to a volumetric flask, and methanol was added until the total volume reached 100 milliliters This procedure yielded a stock solution with a concentration of 1 mg/ml (1000 µg/ml).

Working standards of formazan were prepared by serial dilution of the stock solution with methanol to achieve final concentrations of 0 (blank), 0.5, 1, 2.5, 5, 10, and 20 µg/ml. The necessary amounts of the stock solution were determined by applying the appropriate calculation formula-

$S_1V_1=S_2V_2$

Where,

S1= Stands for the stock solution concentration

V1= Refers to the stock solution amount that needs to be added.

S2= The desired final concentration, and

V2=The total volume for each standard was adjusted to 10 ml.

Each standard solution was thoroughly mixed to ensure complete dissolution of formazan. The optical density (OD) of each standard was then measured at the appropriate wavelength (typically 600 nm) using a spectrophotometer.

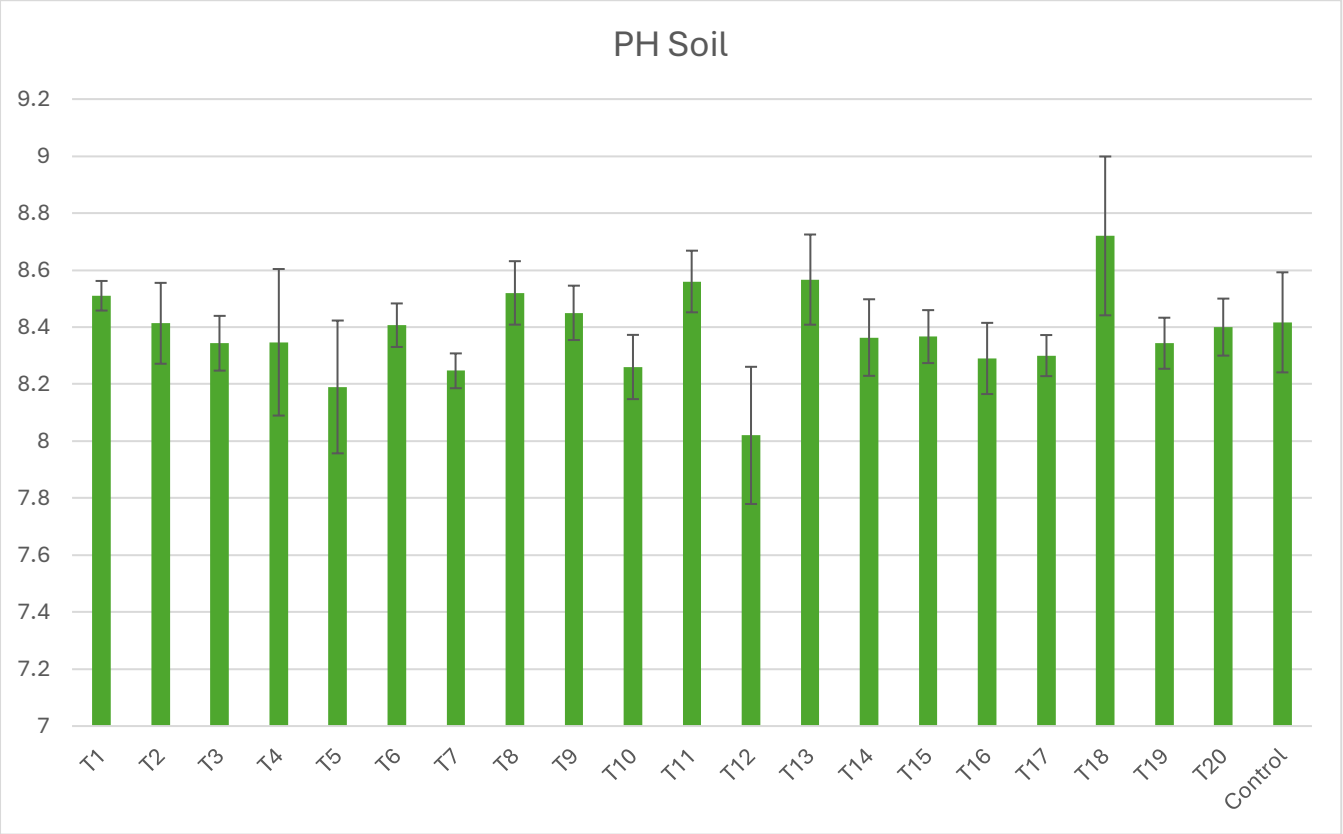
Figure 10: Standard Solutions of Formazan

4. Result

4.1 Soil pH and Electrical Conductivity:

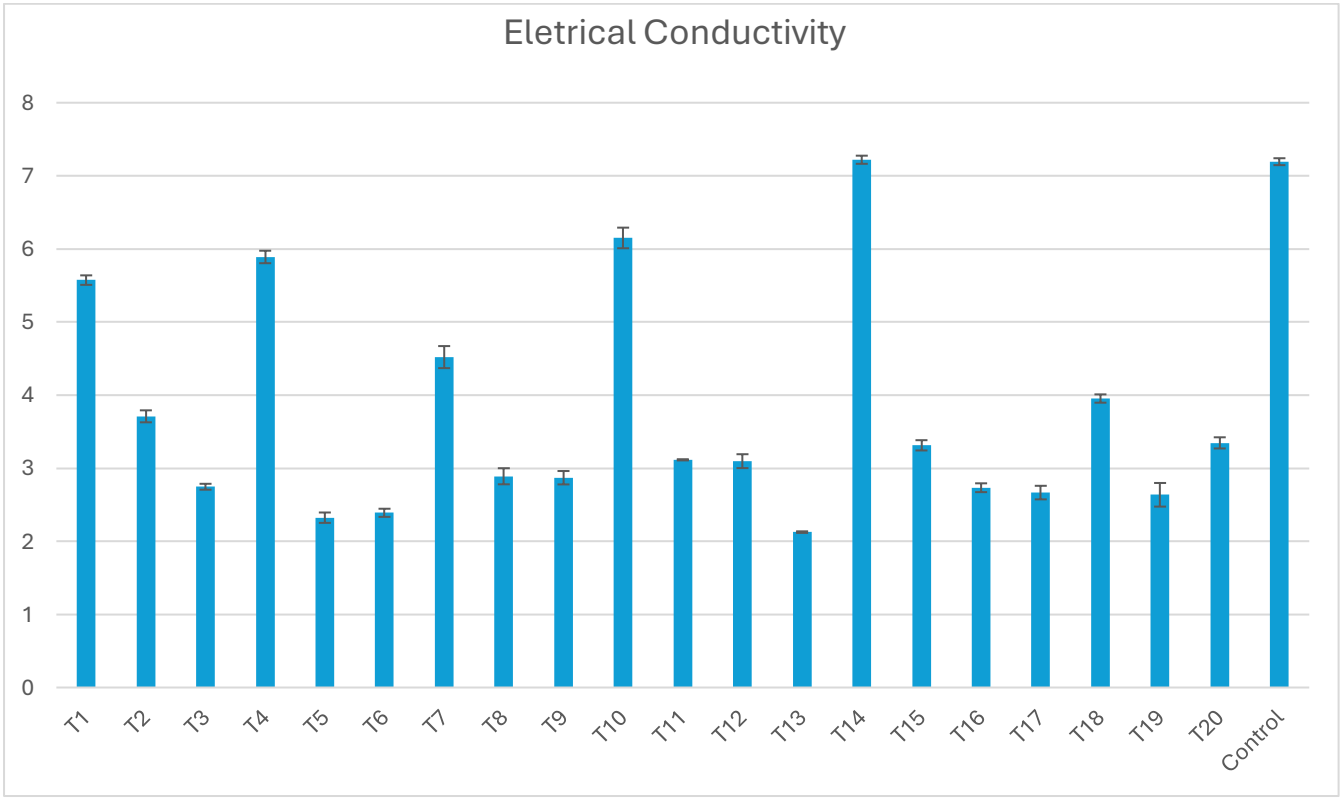
4.1.1 Soil pH

Sample	pH (MEAN)	Standard Deviation
T1	8.51	±0.051961524
T2	8.41	±0.141891978
T3	8.34	±0.096090235
T4	8.34	±0.25716402
T5	8.19	±0.233023604
T6	8.40	±0.076376262
T7	8.24	±0.061101009
T8	8.52	±0.111355287
T9	8.45	±0.09539392
T10	8.26	±0.112694277
T11	8.56	±0.108166538
T12	8.02	±0.240624188
T13	8.56	±0.158219257
T14	8.36	±0.134288247
T15	8.36	±0.092915732
T16	8.29	±0.12489996
T17	8.3	±0.072111026
T18	8.72	±0.278747197
T19	8.34	±0.089628864
T20	8.4	±0.1
Control	8.41	±0.175594229



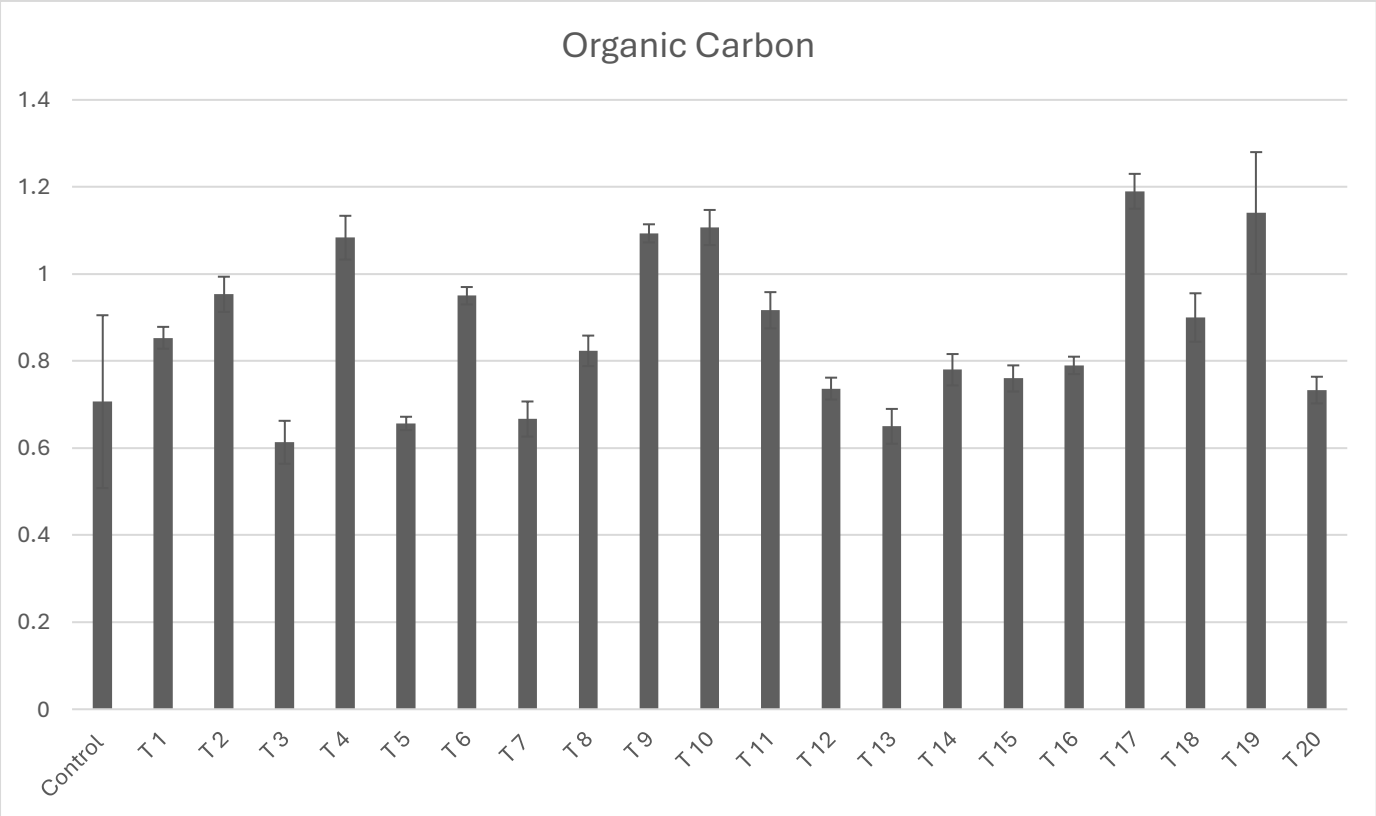
4.1.2 Electrical Conductivity

Sample	EC (MEAN)	Standard Deviation
T1	5.57	±0.065064071
T2	3.71	±0.081853528
T3	2.74	±0.040414519
T4	5.89	±0.085440037
T5	2.32	±0.070945989
T6	2.39	±0.055677644
T7	4.52	±0.150996689
T8	2.89	±0.11
T9	2.87	±0.091651514
T10	6.15	±0.14106736
T11	3.11	±0.007937254
T12	3.09	±0.09504385
T13	2.12	±0.010816654
T14	7.22	±0.055677644
T15	3.31	±0.070237692
T16	2.73	±0.060277138
T17	2.66	±0.092915732
T18	3.95	±0.056862407
T19	2.63	±0.161658075
T20	3.34	±0.076376262
Control	7.19	±0.047258156



4.2 Organic Carbon Content:

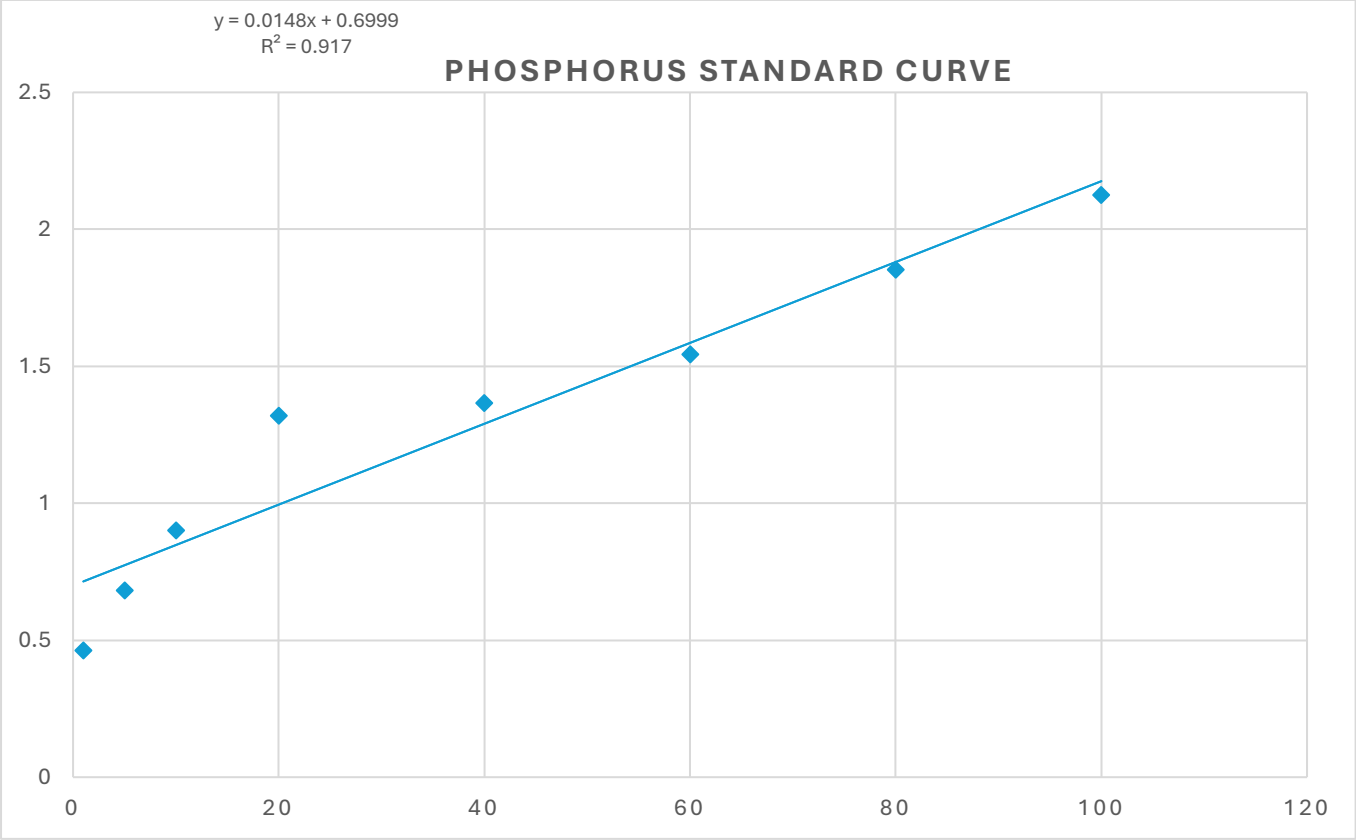
Sample	Available Organic Carbon	Standard Deviation
Control	0.70	±0.19857828
T 1	0.85	±0.025166115
T 2	0.95	±0.040414519
T 3	0.61	±0.049328829
T 4	1.08	±0.05033223
T 5	0.65	±0.015275252
T 6	0.95	±0.02
T 7	0.66	±0.040414519
T 8	0.82	±0.035118846
T 9	1.09	±0.02081666
T 10	1.10	±0.040414519
T 11	0.91	±0.04163332
T 12	0.73	±0.025166115
T 13	0.65	±0.04
T 14	0.78	±0.036055513
T 15	0.76	±0.03
T 16	0.79	±0.02
T 17	1.19	±0.04
T 18	0.9	±0.055677644
T 19	1.14	±0.14
T 20	0.73	±0.030550505



4.3 Available Phosphorus Levels:

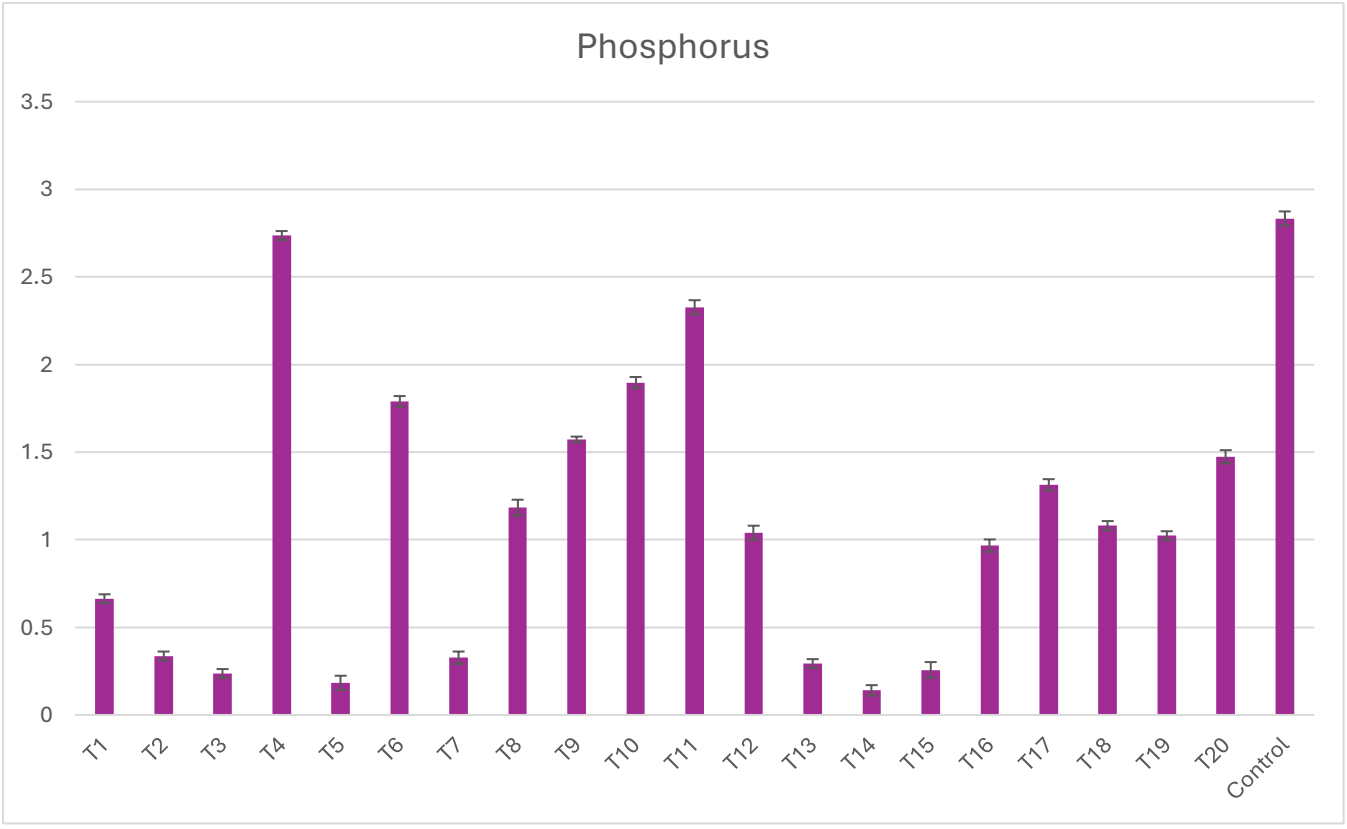
Standard Curve

Concentration (ppm)	OD
blank	0
1	0.464
5	0.684
10	0.902
20	1.32
40	1.367
60	1.544
80	1.855
100	2.127



Available Phosphorus

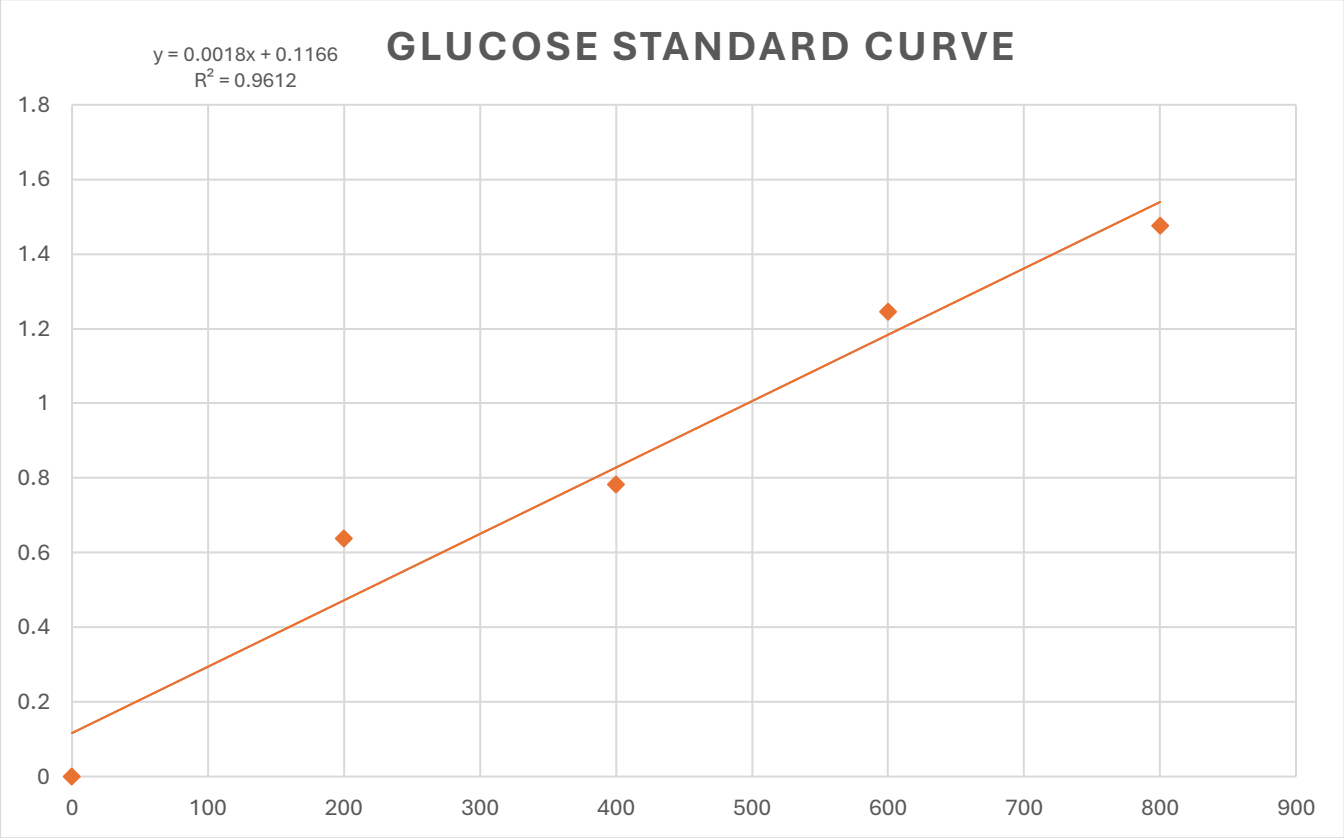
Sample	Phosphorus (mean)	Standard Deviation
T1	0.66	±0.025166115
T2	0.33	±0.025166115
T3	0.23	±0.025166115
T4	2.73	±0.025166115
T5	0.18	±0.040414519
T6	1.79	±0.03
T7	0.32	±0.035118846
T8	1.18	±0.045092498
T9	1.57	±0.015275252
T10	1.89	±0.032145503
T11	2.32	±0.040414519
T12	1.04	±0.04
T13	0.29	±0.025166115
T14	0.14	±0.03
T15	0.25	±0.045092498
T16	0.96	±0.035118846
T17	1.31	±0.032145503
T18	1.08	±0.026457513
T19	1.02	±0.025166115
T20	1.47	±0.037859389
Control	2.83	±0.040414519



4.4 Soil Enzyme Activity:

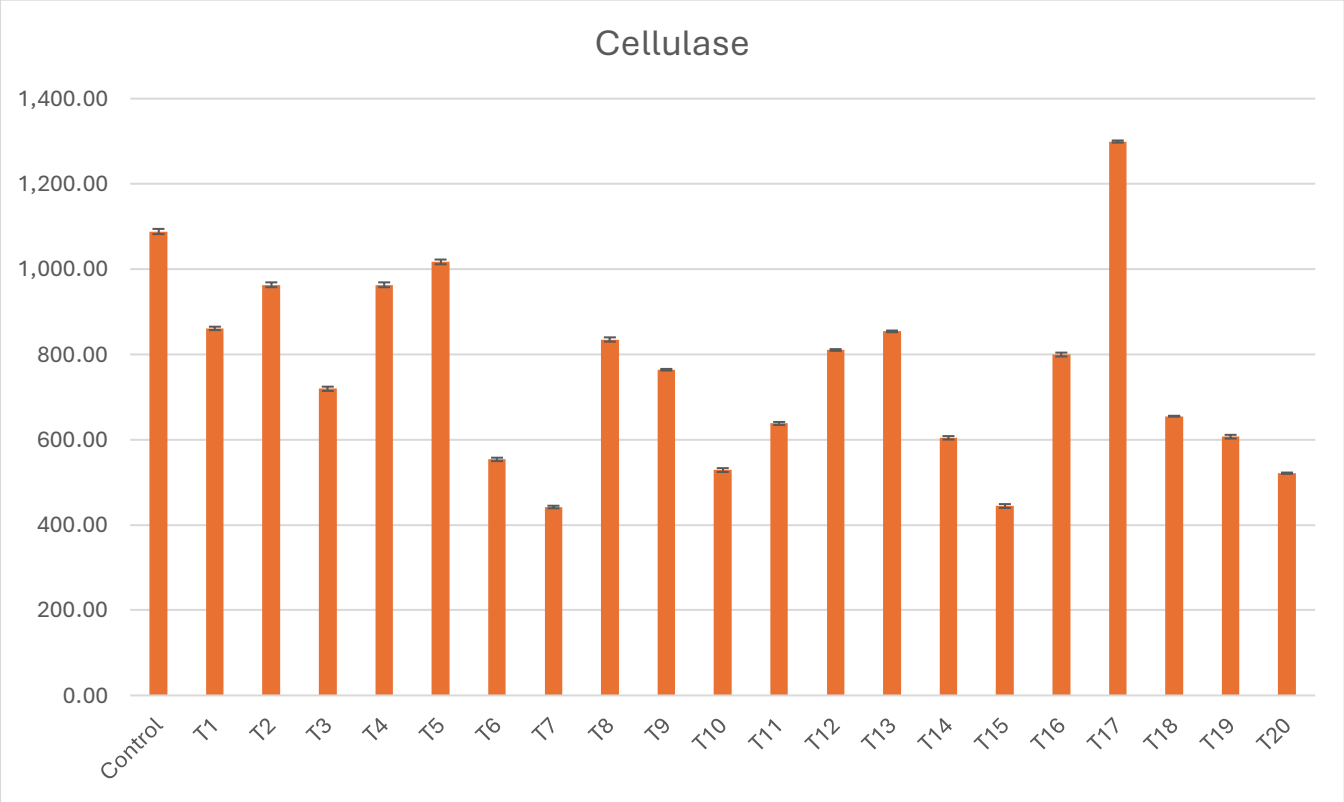
4.4.1 Cellulase Enzyme (Standard Curve)

Glucose (ppm)	OD (540 nm)
0	0
200	0.638
400	0.782
600	1.245
800	1.475



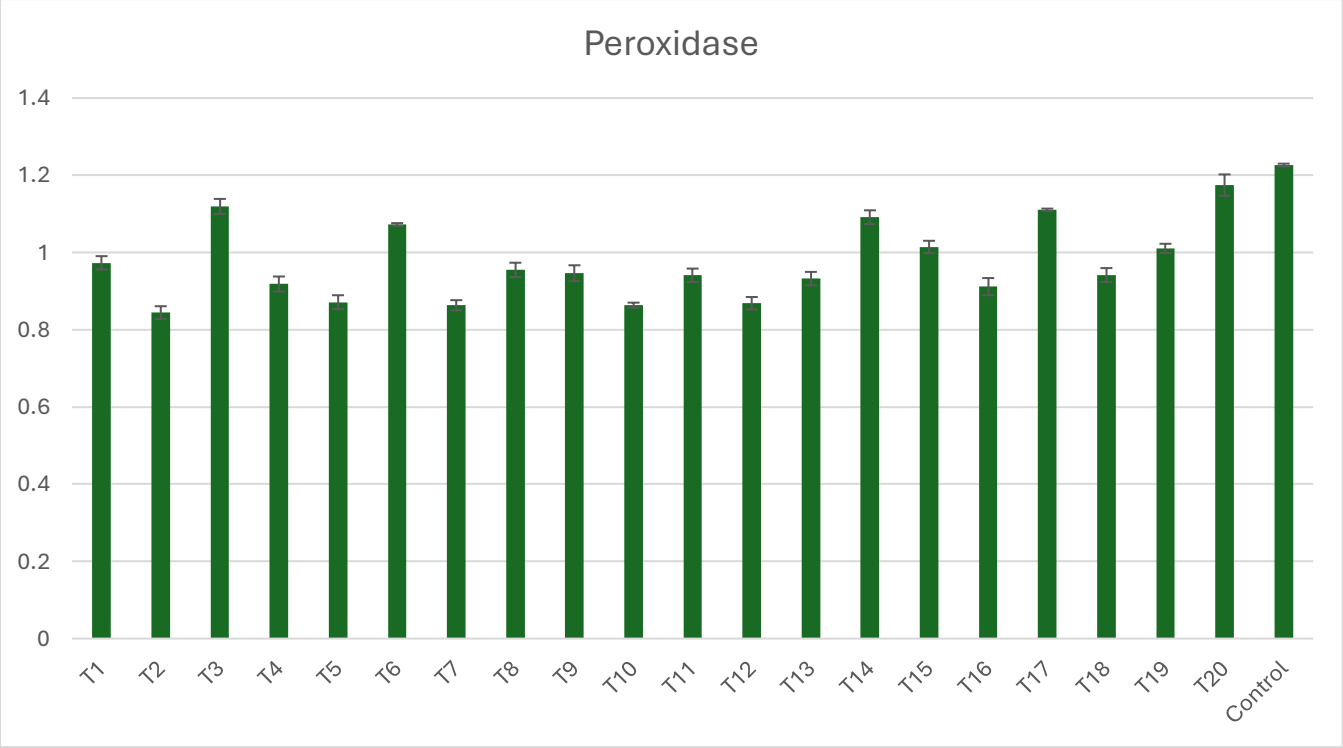
4.4.2 Cellulase Production

Sample	Cellulase Production (MEAM)	Standard Deviation
Control	1,088	±6.110100927
T1	861	±4
T2	963	±5.686240703
T3	719	±5.033222957
T4	963	±5.686240703
T5	1017	±5.567764363
T6	553	±4.041451884
T7	442	±3
T8	835	±5
T9	764	±2
T10	528	±4.509249753
T11	638	±3.605551275
T12	810	±2.081665999
T13	854	±2
T14	604	±4.041451884
T15	444	±4.509249753
T16	799	±4.509249753
T17	1299	±2.516611478
T18	655	±1
T19	607	±4.358898944
T20	521	±1.527525232



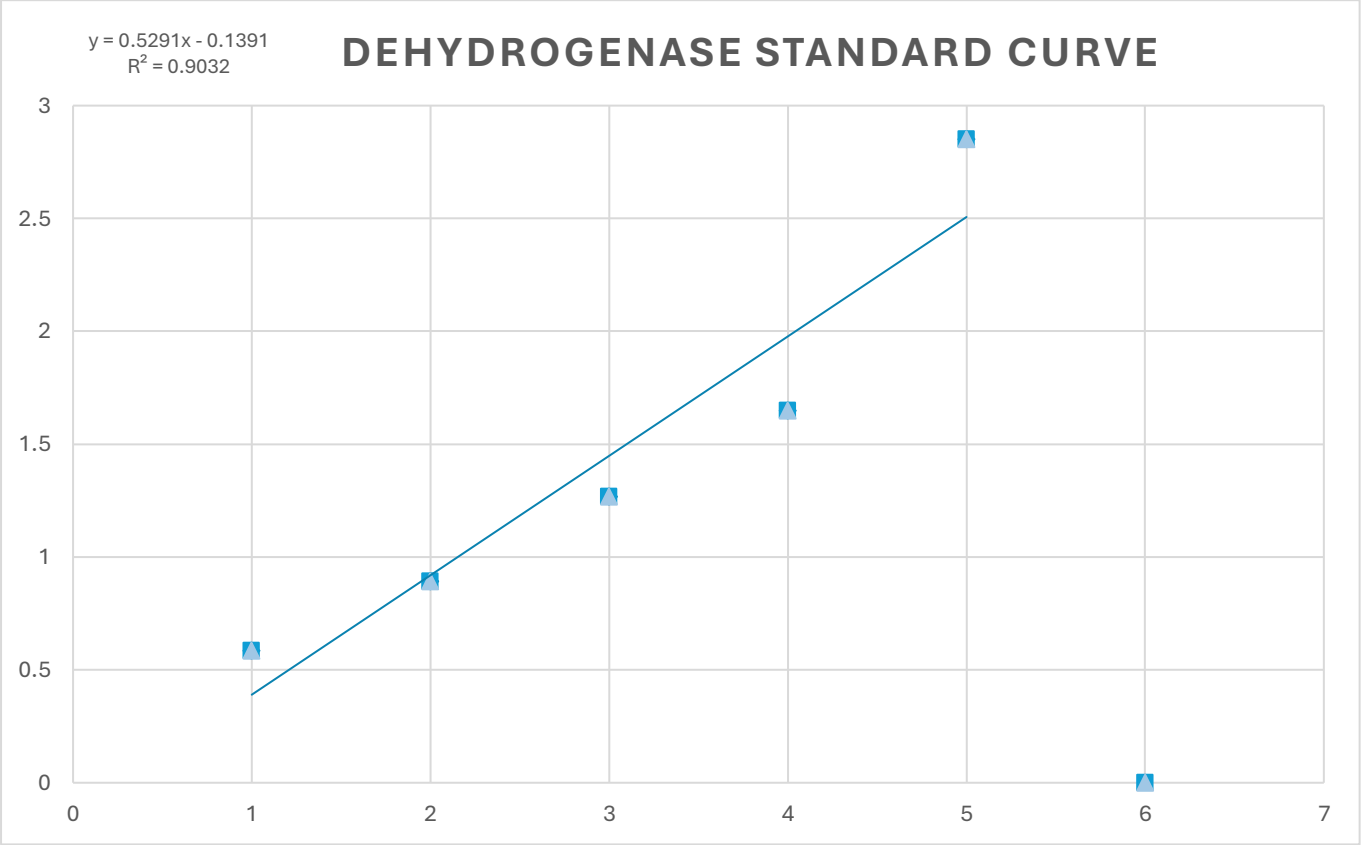
4.4.3 Peroxidase Enzyme:

Sample	Peroxidase (MEAN)	Standard Deviation
T1	0.973	±0.017243356
T2	0.844	±0.016502525
T3	1.119	±0.019502137
T4	0.918	±0.019502137
T5	0.871	±0.018009257
T6	1.072	±0.003511885
T7	0.863	±0.013203535
T8	0.955	±0.018520259
T9	0.946	±0.020599353
T10	0.863	±0.006429101
T11	0.941	±0.017349352
T12	0.868	±0.016072751
T13	0.932	±0.01747379
T14	1.091	±0.018009257
T15	1.014	±0.016010413
T16	0.911	±0.022188586
T17	1.110	±0.00305505
T18	0.941	±0.018009257
T19	1.010	±0.011930353
T20	1.174	±0.027610385
Control	1.226	±0.003511885



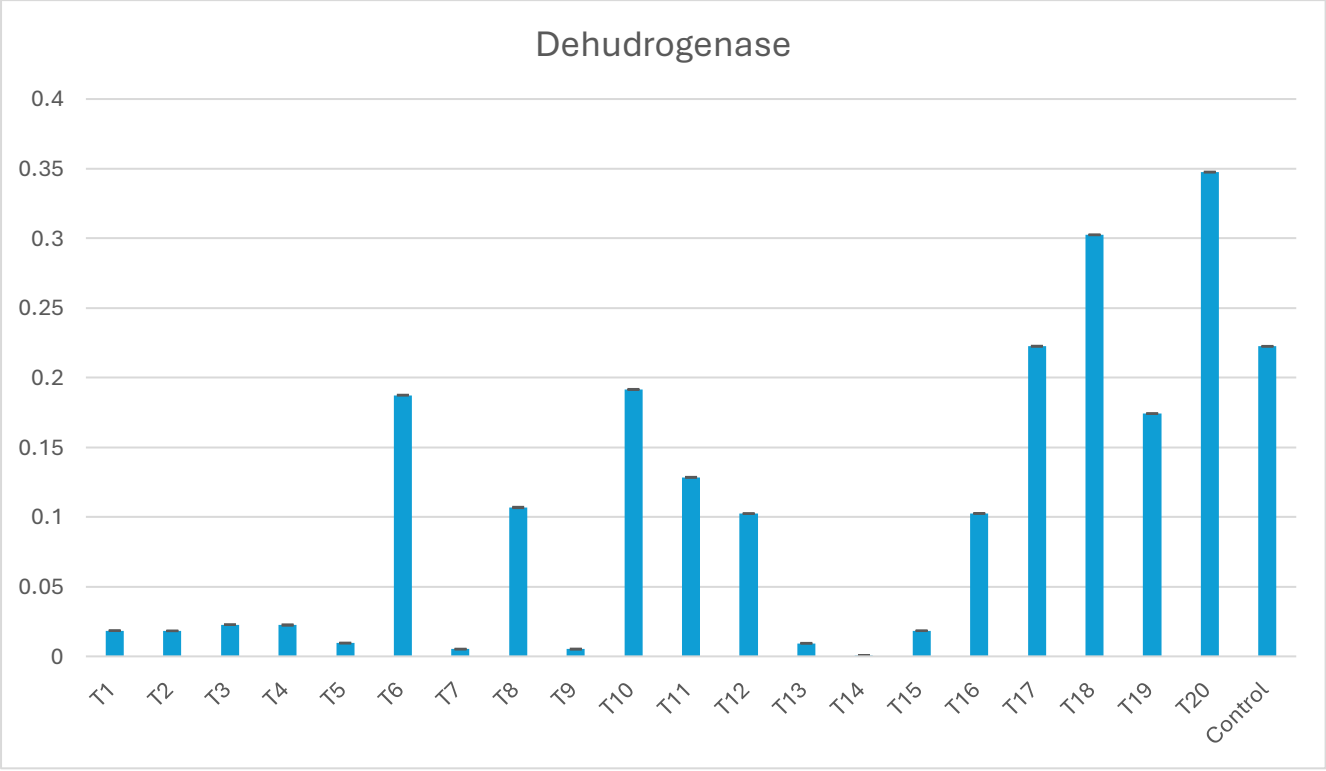
4.4.4 Dehydrogenase (Standard Curve)

Formazan	OD Concentration (µg)
0.5	0.584
1	0.892
2.5	1.266
5	1.647
10	2.852
Blank	0



Formazan Formed

Samples	Formazan formed	Standard Deviation
T1	0.0185	±0.0003
T2	0.0183	±0.00023094
T3	0.0228	±0.000264575
T4	0.0225	±0.000458258
T5	0.0095	±0.000321455
T6	0.1874	±0.000305505
T7	0.0052	±0.0003
T8	0.1068	±0.000450925
T9	0.0052	±0.000360555
T10	0.1915	±0.000351188
T11	0.1284	±0.000351188
T12	0.1025	±0.0003
T13	0.0093	±0.000305505
T14	0.0006	±0.000152753
T15	0.0184	±0.000251661
T16	0.1025	±0.000208167
T17	0.2225	±0.000351188
T18	0.3025	±0.000305505
T19	0.1742	±0.000251661
T20	0.3475	00.000321455
Control	0.2225	±0.0003



Conclusion:

This study demonstrates that tailored microbial treatments can enhance soil health in saline environments while mitigating ecological risks. Key takeaways include:

1. *pH Stability*: The experimental design successfully maintained alkaline conditions, ensuring that pH did not bias other results.

2. *Salinity Reduction*: Treatments like T12 and T14–T17 lowered EC, offering a blueprint for managing salt-affected soils.

3. *Carbon Enrichment*: T11 and T17 significantly boosted organic carbon, reinforcing their role in improving soil fertility.

4. *Phosphorus Control*: Most treatments reduced soluble phosphorus, curbing eutrophication risks—a vital outcome for aquatic ecosystems.

5. *Enzymatic Balance*: Select treatments (T17, T20, T3) preserved or enhanced peroxidase and dehydrogenase activity, critical for stress resilience and nutrient cycling.

The standout performer, T17, excelled in organic carbon accumulation, cellulase activity, and dehydrogenase function, making it a prime candidate for scaling in saline agriculture. Conversely, treatments like T7 and T14, which suppressed enzyme activity, may require reformulation.

Practical Implications

Farmers in regions like the Sundarbans could adopt T17 and T20 to improve soil health without compromising natural defence mechanisms. Policymakers might incentivize these treatments to reduce phosphorus runoff and combat coastal eutrophication.

Future Directions

1. Investigate microbial community shifts under T17 to identify keystone species.

2. field trials conduction help to assess long-term effects on crop yield.

3. Explore synergies between high-performing treatments (e.g., combining T17 and T20).

By bridging microbial ecology and soil science, this work advances sustainable strategies for managing fragile ecosystems, proving that targeted biological interventions can harmonize agricultural productivity with environmental stewardship.

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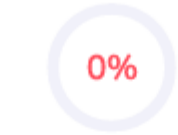

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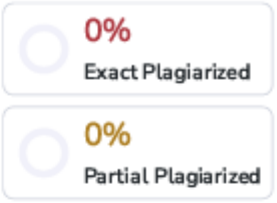

Unique Content

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Discussion

The experimental findings provide critical insights into the interplay between soil treatments, enzymatic activity, and key physicochemical parameters in saline ecosystems. The stability of pH across all treatments (8.02–8.72) underscores the resilience of the system to external interventions, ensuring that alkalinity did not confound other results. This consistency is particularly significant in saline environments like the Sundarbans, where fluctuating pH could disrupt microbial communities and nutrient cycles. The marginal differences between treatments and the control further validate the experimental design, as unintended pH shifts were avoided.

Electrical conductivity (EC), a proxy for salinity, revealed a clear trend: most treatments (e.g., T12, T14–T17) reduced EC compared to the control. This suggests that specific bacterial isolates or amendments mitigated salt stress, a common challenge in coastal agriculture. Lower EC values correlate with improved soil structure and plant growth, as excessive salinity inhibits water uptake and nutrient absorption. The success of treatments like T12 and T14–T17 highlights their potential for rehabilitating salt-affected soils, though long-term field studies are needed to confirm these effects.

Organic carbon content, a linchpin of soil fertility, showed marked improvements in treatments T7, T11, and T17. These increases likely stem from microbial activity enhancing organic matter decomposition or root exudate production. For instance, T17's dual role in boosting organic carbon and cellulase activity (via glucose release) suggests a synergistic relationship between carbon cycling and enzyme efficiency. Conversely, treatments like T1 had minimal impact, emphasizing the need for targeted interventions.

Phosphorus dynamics revealed an environmental success story: most treatments reduced soluble phosphorus relative to the control (2.83 mg/L). Lower phosphorus levels mitigate eutrophication risks, a critical concern in waterlogged ecosystems. However, the outlier T4 (2.73 mg/L) warrants scrutiny—was this due to microbial phosphate solubilization or experimental error? Further replication could clarify this anomaly.

Enzymatic profiles offered a window into microbial metabolism. Peroxidase activity, crucial for oxidative stress response, remained robust in T3, T20, and others, indicating that specific bacteria preserved soil defense mechanisms. In contrast, dehydrogenase activity—a metabolic bellwether—varied dramatically. The standout performance of T20 (0.3476 formazan) and T17 (0.2226) contrasts with near-zero activity in T7 and T14, suggesting that some treatments either enhanced microbial vitality or introduced inhibitory agents. The control's high dehydrogenase activity (0.2225) sets a benchmark for evaluating treatment efficacy.

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Abstract

The study concentrates on the enzymatic activity of the bacterial enumerated soil of the Indian Sundarban and also the organic carbon measurement. The soil samples were collected from the different location within Nimpith (Sundarban).

This research explored how different soil treatments could improve soil health in salty environments. Throughout the study, the focus was on several important soil properties. The pH values across all experimental groups remained within a moderately alkaline range (8.02–8.72) and showed minimal variation, indicating that the treatments did not significantly alter soil pH. Electrical conductivity was generally reduced in the treated groups compared to the control, reflecting a decrease in soil salinity. Which is essential for supporting healthy plant growth.

Several treatments led to a marked increase in soil organic carbon content, suggesting enhanced soil fertility and improved potential for sustainable crop production.

Phosphorus levels were consistently lower in treated soils, which may help mitigate the risk of nutrient runoff and environmental eutrophication.

Most treatments resulted in peroxidase and glucose enzyme activities that were comparable to or lower than the control, with some treatments demonstrating enhanced enzymatic activity related to cellulose degradation.

Overall, the study highlights that thoughtful soil management can help counteract the negative effects of salinity, leading to healthier soils and more sustainable farming in challenging conditions.

Improved soil conditions can support a wider variety of crops and beneficial soil organisms, fostering a more balanced and diverse ecosystem.

The promising results from certain treatments suggest that further research and application of salt-tolerant bacteria could help reclaim and improve saline soils in other regions, making previously unproductive land viable for agriculture.

Continued use of organic amendments and targeted microbial treatments could gradually restore degraded soils, making them more fertile and resilient to future environmental stresses.

With the threat of rising soil salinity due to climate change, these approaches offer practical solutions for sustaining food production. Future studies could focus on large-scale field trials, economic assessments, and developing easy-to-use products for farmers, ensuring these benefits reach more communities

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Soil is much more than just earth beneath our feet—it's a vibrant blend of minerals, moisture, air, and a whole community of living organisms, all working together to support life above ground. All these ingredients come together to create the foundation for healthy plant life and thriving ecosystems. It forms the uppermost layer of the earth, providing habitat and shelter for various vibrant organisms and supporting numerous ecological communities. The organic matter makes only 5%, filling voids, water and air takes up to a 50% and minerals taking the lead with 45%.

Soil is made through weathering and erosion, along with the decomposition of organic material due to climate, organisms and topography (Williams, Friedl 2023). It serves a critical role in water storage, agriculture, nutrient cycling and modifying the atmosphere.

The soil is home to a wide variety of tiny living organisms, including bacteria, fungi, archaea, and protozoa. All of these are known as microbes.

An astonishing amount of them, up to billions, are contained in just 1 gram of soil.

Microbes also help in soil fertility through recycling nutrients, decomposing residues and breaking down organic matter.

The rhizosphere or the placed around plant roots is vital because, it aids the plant in further growth, recovery and tolerance. Plants symbiotically team up with phytomicrobes, augmenting growth and tolerance against stress and infact disease.

Active microbes and healthy oil boost agricultural output by improving nutrient availability, making the soil more fertile and warding off pests and disease. Soil serves as much more than just a medium for plant roots; it is a vital resource that supplies plants with the nutrients and water they need to thrive. The structure of soil offers a stable foundation where plant roots can firmly anchor themselves and extend outward as they seek water and nutrients. This anchorage not only stabilizes the plant but also enables roots to absorb essential minerals and moisture, both of which are crucial for initiating and sustaining key metabolic functions.

These processes are fundamental to plant growth, energy production, and longterm survival.

In this way, soil acts as both a support system and a life-sustaining source, directly influencing the health and vitality of plants.

Soil gives nutrients, waters, and a stable place where roots can fixate and work optimally for development and sustenance. Fertile soil inhabited by microorganisms is extremely beneficial for agricultural production as it increases the nutrient supply and inhibits pathogens and pests. Microbial activities help to mitigate climate change through sequestering carbon in soil. (Front. Soil Sci., 11 April 2022 Sec. Soil Biogeochemistry & Nutrient Cycling)

Soil preserves temperature, controls flooding, and minimizes erosion which helps whole ecosystems. It also stores water, filters it, and purifies it as well controlling water movement to aquifers and rivers while reducing contamination.

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Total Words: 510 **Total Characters:** 3279 **Plagiarized Sentences:** 0 **Unique Sentences:** 24 (100%)

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3. Material And Methods

3.1 Study Area and Procedure for applying bacterial Solution to soil samples:

Materials Required
- Pots (sterile)
- Soil samples from Nimpith
- Bacterial strains
- Greenhouse chamber
- Water (distilled)
- Grinder (for soil grinding)
- Incubator (set at 37°C)

Experimental Procedure Soil
Preparation:
Soil samples were first collected from Nimpith, a small town positioned in the delta region of the Sundarbans, which lies within the extensive Ganges Delta. The soils in this area are generally a mix of clay, sand, and silt, and are regularly influenced by tidal flooding and varying levels of salinity due to their proximity to both river and sea. With its low elevation and closeness to the mangrove ecosystem, Nimpith serves as an ideal location for examining the unique soil properties that define the Sundarbans delta.
T
The soil samples were gently sifted to eliminate any stones and unwanted debris. Afterward, the soil was mixed thoroughly to achieve a uniform consistency throughout. Each experimental pot was filled with an equal amount of soil, specifically 200 grams per pot, to maintain consistency across all treatments.

Preparation of Bacterial Solution:
Bacterial strains were cultured in a suitable broadcast medium until they reached a cell density

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Soil pH showed whether the soil was more on the acidic or alkaline side, based on how many hydrogen ions were present in the soil's water. This was an important factor because it affected how easily plants could take up the nutrients they needed to grow well.

To find out the soil's pH, samples were mixed with water, and the amount of hydrogen ions in the mixture was measured. Knowing the pH helped to understand how well the soil could support different kinds of plants.

Sample Preparation
A 10-gram portion of air-dried, sieved soil was weighed and transferred into a clean 50 mL beaker. Subsequently, 25 mL of distilled water was added to the soil, maintaining a consistent ratio for all samples.

Mixing and Equilibration
The mixture was stirred to ensure even dispersion of soil particles. The mixture was gently stirred at regular intervals for a total of 30 minutes. After mixing, the beaker was left undisturbed for one hour to allow the soil particles to settle and the solution to reach equilibrium.

Calibration of pH Meter
Before taking any measurements, the pH meter was standardized with buffer solutions having pH values of 4.0, 7.0, and 9.2. The electrode was rinsed with distilled water between each calibration and sample measurement to avoid contamination.
pH Measurement
Once the suspension had settled, the pH electrode was carefully inserted into the supernatant above the settled soil. The pH reading was allowed to stabilize before being recorded for each sample.

Electrode Maintenance
After each measurement, the electrode was thoroughly rinsed with distilled water to maintain accuracy for subsequent samples.
Electro conductivity Test:
Principle
Electrical conductivity (EC) indicates how well a soil solution carries electricity, revealing the amount of dissolved salts affecting its salinity
Reagents and Calibration
For calibration purposes, a 0.01M potassium chloride (KCl) solution was employed as the standard reference. This solution typically exhibited an electrical conductivity value in the range of 1.412 to 1.413 dS/m at a temperature of 25°C. To prepare the 0.01M KCl standard, 0.746 grams of potassium chloride were accurately weighed and dissolved in one litre of distilled water. Prior to each series of measurements, the conductivity meter was calibrated using this standard solution to ensure precise and reliable results.

Procedure
A 10-gram sample of air-dried soil was accurately weighed and placed into a clean beaker.

To this, 50 milliliters of distilled or deionized water were added, maintaining a soil-to-water ratio of 1:5.

The mixture was then shaken well for about 30 minutes to make sure all the soluble salts in the soil dissolved completely.

The suspension was allowed to settle undisturbed for 4 hrs to obtain a clear soil extract for analysis.
Prior to measurement, the electrical conductivity (EC) meter was calibrated with the 0.01M KCl standard solution at 25°C to ensure accuracy.
The electrode was thoroughly rinsed with distilled water before being carefully immersed in the clear soil extract, taking care to avoid disturbing

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The mixtures were then titrated with 0.5 N ferrous ammonium sulfate (FAS) solution, added slowly until the blue color transitioned to green, indicating the endpoint.
A blank titration was also performed using the same procedure but without the soil sample, to ensure accuracy and allow for correction of reagent volumes.

Volume of FAS used for the sample titration and compared it to the blank titration (without soil). Find out how much organic carbon is present by using this formula: %organic carbon= 10× (Vblank −Vsample) /blank × 0 .003 ×100 /weight of soil (1g)

Vblank= volume of FAS for blank (mL)

Vsample=volume of FAS for sample (mL)Each millilitre of 1N $K_2Cr_2O_7$ corresponds to 0.003 grams of carbon that has been oxidized.

The Walkley-Black method was used to determine soil organic carbon by oxidizing the organic carbon present in the soil with chromic acid, which was produced from the reaction between potassium dichromate and sulfuric acid. After the oxidation reaction, the remaining unreacted dichromate was measured by back-titration with ferrous ammonium sulfate, using diphenylamine as an indicator to identify the endpoint. This method enabled the measurement of the amount of oxidizable organic carbon present in the soil samples.

3.4 Estimation of Available Phosphorus:

To measure available phosphorus in neutral to alkaline soils, the Olsen method is widely used. This approach starts by shaking a soil sample with a sodium bicarbonate solution (0.5 M, pH 8.5), which helps release phosphorus that plants can use by breaking its bonds with soil particles. After extraction, the filtered solution is treated with ammonium molybdate in an acidic medium, causing a chemical reaction that forms a blue-coloured compound. Adding stannous chloride further intensifies this blue color, making it easier to measure the phosphorus content with a spectrophotometer. The richer and deeper the blue color that appears in the solution, the more phosphorus is available in the soil sample. This color change makes it easy to estimate the phosphorus content, as a stronger blue indicates a higher amount of phosphorus that plants can use.

Materials Used

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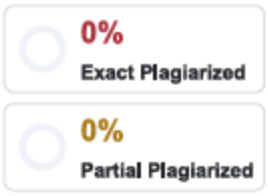

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A primary phosphorus stock solution was prepared by dissolving 0.2195 g of potassium dihydrogen phosphate (KH_2PO_4, dried at 105°C) in distilled water and diluting to 1 litre. This solution contained 50 mg per Litre (50 ppm P). To prepare a secondary stock solution with a concentration of 100 ppm, I took 2 milliliters of the primary stock solution and carefully diluted it to a final volume of 1 litre using distilled water. This process ensured that the secondary solution reached the desired concentration.

Preparation of Working Standards by Serial Dilution
A series of working standard solutions with concentrations of 1, 5, 10, 20, 40, 60, 80, and 100 ppm were prepared from the 100 ppm secondary stock solution using serial dilution.
Each standard was prepared in a clean 10 mL volumetric flask. The required volume of the 100 ppm stock solution was pipetted into the flask, and distilled water was added to bring the total volume to 10 mL. Each solution was mixed properly.

Each standard was prepared in a clean 10 mL volumetric flask. The required volume of the 100 ppm stock solution was pipetted into the flask, and distilled water was added to bring the total volume to 10 mL.

Color Development and Measurement
In a 25 mL volumetric flask 5 ml of each standard solution was transferred. To this, 10 mL of ammonium molybdate reagent and 1 mL of freshly prepared stannous chloride solution were added.

The solution was then carefully topped up to the final volume with distilled water and mixed well to ensure uniformity. After mixing, the samples were left undisturbed for 10 minutes, allowing the blue color to fully develop before any measurements were taken.

The OD of the solution was measured at 880 nm wavelength by a spectrophotometer.

Assay of Soil Enzymatic Activity:

- Cellulase
- Dehydrogenase
- Peroxidase

Soil Enzyme Activity as an Indicator of Soil Health and Fertility

Soil enzyme activity is fundamental to the decomposition of organic matter and the cycling of nutrients, processes that are integral to maintaining soil quality and fertility. These enzymes, which originate primarily from soil microorganisms, plant roots, and decomposing organic residues, catalyze the breakdown of complex organic substrates into simpler compounds. This transformation not only facilitates the release of essential nutrients for plant uptake but also drives the biogeochemical cycling of key elements such as carbon, nitrogen, and phosphorus.
One of the most remarkable things about soil enzymes is how quickly they react when something in their environment changes-whether it's a shift in farming practices or fluctuations in weather conditions.
As a result, the measurement of soil enzyme activities has emerged as a sensitive and early indicator of shifts in soil health and fertility, often preceding detectable changes in other soil properties.